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Interlaboratory Evaluation of an Off-Line SFE/IR Method for Determination of Petroleum Hydrocarbons in Solid Matrices

Project Report

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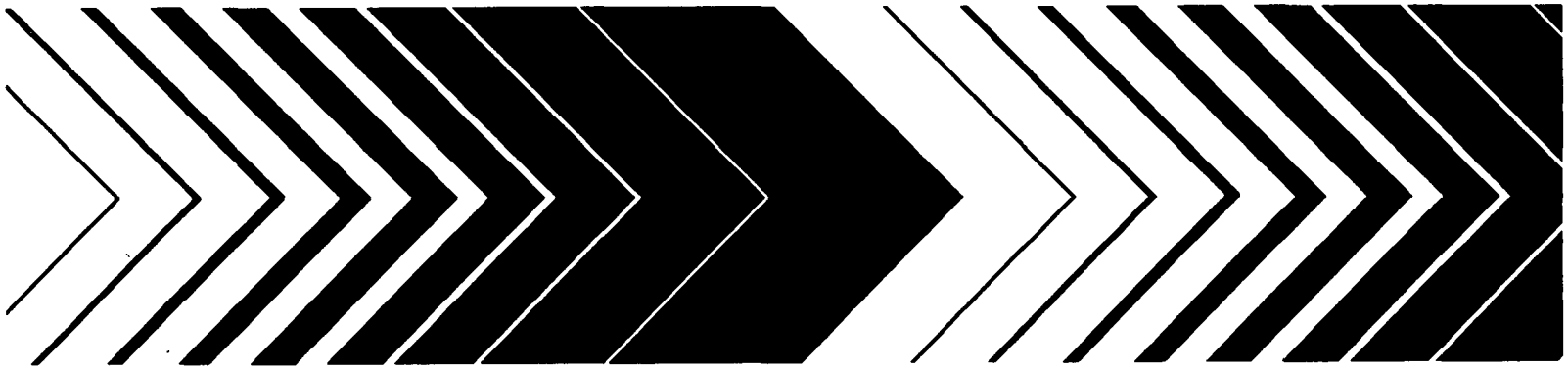

INTERLABORATORY EVALUATION OF AN OFF-LINE SFE/IR METHOD FOR DETERMINATION OF PETROLEUM HYDROCARBONS IN SOLID MATRICES

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PREFACE

This is the final report for Work Assignment 2-7, EPA Contract 68-C1-0029, conducted at Midwest Research Institute, California Operations. The project was directed by Dr. Viorica Lopez-Avila.

This report was written by Dr. Viorica Lopez-Avila. Technical support for the project was provided by Richard Young and Robert Kim.

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We gratefully acknowledge their contributions.

ABSTRACT

A collaborative study was conducted, with Midwest Research Institute, California Operations (MRI-CO), as the lead laboratory, and with 13 additional volunteer laboratories participating, to determine the method accuracy and precision of EPA SW-846 draft Method 3560. This method describes the extraction of petroleum hydrocarbons from solid matrices with supercritical carbon dioxide at 340 atm and 80°C for 30 min (dynamic; flow rate 1 to 2 mL/min as liquid) and collection of the extracted materials in 3 mL tetrachloroethylene. The extracts generated by the participating laboratories were shipped to MRI-CO for analysis by infrared spectrometry (draft Method 8440). The collaborative study was based on the AOAC International blind replicate design with balanced replicates. Four soil samples were to be extracted in triplicate. Three of these samples were standard reference materials with TPH contents of 614, 2050, and 32,600 mg/kg, respectively. The fourth sample was a clay soil spiked with motor oil at 10,000 mg/kg. Each of the four samples was extracted by the laboratories in triplicate with supercritical carbon dioxide, and the extracts were analyzed by MRI-CO on two different infrared spectrometers. In addition, each of the participating laboratories extracted a sample of the unspiked clay soil, clay soil spiked with both corn oil and reference oil (a mixture of n-hexadecane, isooctane, and chlorobenzene) at 1,000 mg/kg each, and clay soil spiked with motor oil at 10,000 mg/kg and brought to a water content of 30 percent (the individual laboratories then had to add anhydrous sodium sulfate). These latter three samples were extracted only once. They were included in the study to address possible cross-contamination in the supercritical fluid extraction system (the laboratories were asked to extract the unspiked clay soil right after extraction of a clay soil sample spiked at 10,000 mg/kg with motor oil), the presence of interferences such as oily materials, and the effect of water content on extraction efficiency.

After outlier removal (using both the Cochran and the Grubbs tests), we calculated the mean concentration, repeatability standard deviation, reproducibility standard deviation, repeatability relative standard deviation, and reproducibility relative standard deviation for each of the four matrices extracted in triplicate. In addition, the relationship between the measured petroleum hydrocarbon contents of these samples and the true concentrations was established.

The results from the triplicate analyses of the four materials show that the method accuracy (percent recovery) for petroleum hydrocarbon concentrations ranging from 614 to 32,600 mg/kg was 83 percent; the mean recoveries of petroleum hydrocarbons from each of the four matrices ranged from 78 to 107 percent for the analyses performed with a Perkin-Elmer FTIR spectrometer, and from 76 to 101 percent for the analyses performed with a Buck-Scientific IR spectrometer. The differences of the results from the two instruments on a sample-by-sample basis were less than 17 percent for the total petroleum hydrocarbon determinations. The interlaboratory method precisions ranged from 17 to 45 percent for analyses on the Perkin-Elmer FTIR system and from 17 to 48 percent for analyses on the Buck-Scientific IR system; the intralaboratory method precisions ranged from 12 to 17 percent for analyses on the Perkin-Elmer FTIR system and from 11 to 18 percent for analyses on the Buck-Scientific IR system. Method accuracy and precision data are presented for the five laboratories that used the Isco supercritical fluid extraction systems and for the seven laboratories that used different supercritical fluid extraction systems, all having extraction vessels of 3.5-mL volume or less.

Analysis of variance (ANOVA) was used to separate (mathematically) the total variation of the experimental measurements into an intralaboratory portion and an interlaboratory portion, with a corresponding split of the total number of degrees of freedom. The ANOVA results indicate that, as expected, the variation from laboratory to laboratory was greater than that attributed to the analytical error displayed within laboratories. The matrix and operational parameters, such as flow rate, size of the extraction vessel, extraction vessel design and orientation, mode of collection of the extracted material, and temperature of the collection solvent/trap all seemed to be important.

The recoveries from the wet clay soil samples mixed with sodium sulfate were above 30 percent for only two laboratories, and eight laboratories had recoveries below 8 percent. Separate experiments conducted at MRI-CO showed that addition of anhydrous magnesium sulfate and Hydromatrix (diatomaceous earth) raised the recoveries to 72 percent or higher for samples containing not more than 20 percent water. When samples contained more than 20 percent water, they had to be mixed with anhydrous magnesium sulfate and allowed to equilibrate for several hours (preferably overnight in sealed containers and at 4°C to minimize losses of volatile petroleum hydrocarbons) before extraction with supercritical carbon dioxide.

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SECTION 1

INTRODUCTION

Widespread use of chlorofluorocarbons and other harmful organic solvents in environmental testing laboratories is of major concern to the U.S. Environmental Protection Agency (EPA). Therefore, EPA's Environmental Monitoring Systems Laboratory in Las Vegas, NV, started the development of supercritical fluid extraction (SFE) methods that replace those harmful organic solvents with supercritical carbon dioxide or carbon dioxide modified with small amounts of methanol or other innocuous organic solvents as extractants. The first SFE method, SW-846 Method 3560, was recently proposed (1, 2). The proposed method describes extraction of petroleum hydrocarbons with supercritical carbon dioxide at 340 atm and 80°C for 30 min (dynamic), with a carbon dioxide flow rate of 1 to 2 mL/min as liquid, or 500 to 1,000 mL/min as decompressed gas. Depending on the extraction system used, the stream of carbon dioxide containing the extracted materials passes either through a collection vial containing 3 mL tetrachloroethylene (also known as perchloroethylene, PCE), or through an adsorbent trap. The technique is simple, requiring approximately 30 min for the extraction of a 2- to 5-g solid sample, and 10 min for extract cleanup (to remove polar organic compounds) and analysis by infrared (IR) spectrometry. The results of a single-laboratory evaluation of this SFE method indicated that its performance is equivalent to the Soxhlet extraction method using Freon-113 as extractant, its accuracy is 80 percent or better, and its precision is $\pm$ 20 percent (1, 2).

A collaborative study of Method 3560 was conducted by 14 laboratories, with Midwest Research Institute, California Operations (MRI-CO), as the lead laboratory, and with 13 additional volunteer laboratories participating. The goal of this study was to determine the method accuracy and precision of draft Method 3560 (Supercritical Fluid Extraction of Petroleum Hydrocarbons) when used at different laboratories using instrumentation from different manufacturers.

The criteria for selecting the laboratories included availability to the laboratories of commercial SFE systems that could be clearly described by the participants, willingness to perform the extractions within a month after sample receipt, previous experience of the prospective collaborators with the SFE technique in general, and willingness to participate as a volunteer.

Subsequent sections of this report present the conclusions and recommendations from this study, the experimental details, and the results. The proposed draft protocols for SFE (Method 3560) and for the total recoverable hydrocarbon determination by IR (Method 8440) are included as Appendices A and B, respectively. The list of instructions sent to the collaborating laboratories is included as Appendix C.

SECTION 2

CONCLUSIONS

The results of this collaborative study, conducted with 14 laboratories, indicate that the proposed EPA SW-846 Method 3560 (when used with EPA Method 8440) has an accuracy of 82.9 percent (this is the overall method recovery of petroleum hydrocarbons from samples containing from 614 to 32,600 mg/kg TPHs). The interlaboratory method precisions ranged from 17.3 to 45.4 percent relative standard deviation for PE-FTIR analyses and from 16.7 to 47.9 percent for BSci-IR analyses; the intralaboratory method precisions ranged from 11.5 to 17 percent for PE-FTIR analyses and from 11.1 to 18.2 percent for BSci-IR analyses.

The matrices used in this study were three standard reference soils and clay soil spiked with TPHs; the TPH recoveries for these samples, which were homogeneous and dry (with the exception of the clay soil that had a water content of 10.6 percent), were greater than 75 percent. However, when the clay soil was mixed with additional water to bring its water content to 30 percent, followed by extraction by SFE, less than 38 percent recovery was achieved. Results from additional experiments performed with anhydrous magnesium sulfate and diatomaceous earth as drying agents indicated that samples containing 20 percent water or more need to be mixed with anhydrous magnesium sulfate and allowed to equilibrate for several hours (preferably overnight in sealed containers and at 4°C to minimize losses of volatile petroleum hydrocarbons) before extraction by SFE.

Contamination of the SFE system is likely, especially when high-contamination samples are extracted; the analyst must take precautions to minimize coss-contamination of extracts.

The participating laboratories generally stayed within the guidelines of the instructions provided by MRI-CO; however, variations in the carbon dioxide flow rate, the extraction vessel dimensions, design, and orientation, the mode of collection of the extracted material, and the temperature of the collection solvent/trap were unavoidable because the various SFE systems were not identical in design. Therefore, it is not surprising that the variation from laboratory to laboratory was greater than that attributed to the analytical error displayed within laboratories. Nonetheless, the participating laboratories did a good job; when the data were subjected to a software program from the Association of Official Analytical Chemists (AOAC), data from only one laboratory were rejected on two of the matrices.

SECTION 3

RECOMMENDATIONS

On the basis of this interlaboratory method validation study, the Office of Solid Waste may want to consider Method 3560 for incorporation in the SW-846 methods manual.

The specific recommendations from the participating laboratories are listed below:

- The temperature of the collection solvent should be kept at 0 to 5°C to minimize losses of volatile organics.

- Laboratory 02 reported that all glass wool in their laboratory was contaminated with petroleum hydrocarbons. Therefore, they recommended that glass wool be washed and dried in a muffle furnace before use.

- Laboratory 02 recommended that glass wool be replaced with a drying agent (magnesium sulfate, diatomaceous earth) to "protect" the frits and fill the void volume of the extraction vessel.

- Laboratory 13 recommended that precleaned sand be used to fill the void volume of the extraction vessel.

- Laboratories 05 and 13 recommended that the direction of carbon dioxide flow be recorded. Furthermore, they stated that the carbon dioxide flow from top to bottom of the extraction vessel is superior to flow from bottom to top when the extraction cell is not full.

- The forms used by the laboratories in reporting the operating conditions (Appendix C) were very helpful, and we recommend that these forms be used to record the SFE conditions.

The method presented here is not suitable for the extraction of hydrocarbons from gasoline-contaminated soil samples because of poor collection efficiencies of the volatile hydrocarbons present in gasoline. Improvements in the collection method, such as trapping onto an adsorbent trap held at -10°C, followed by rinsing of the trap with an organic solvent (PCE) and analysis by gas chromatography with infrared detection, is recommended for future studies.

SECTION 4

EXPERIMENTAL

COLLABORATIVE STUDY

Study Design

The study was based on the AOAC's blind replicate design with balanced replicates for the collaborative evaluation of precision and accuracy of an analytical method (3,4). In our study, four soil samples were to be extracted in triplicate. Samples 1, 2, and 3 were standard reference materials that had been certified for TPHs by the modified Method 418.1(5); their TPH contents were 614, 2050, and 32,600 mg/kg, respectively. The fourth sample was a clay soil that we spiked with motor oil at 10,000 mg/kg. The samples, including their code numbers and TPH levels, are described in Table 1.

Each laboratory received 10-g portions from matrices 1, 2, and 3, and three 3-g portions of clay soil spiked with motor oil at 10,000 mg/kg. The spiked clay soil samples were identified as samples 4, 6, and 7. In addition, they received the unspiked clay soil, identified as sample 5, and clay soil samples spiked with corn oil/reference oil and motor oil (identified as samples 8 and 9 respectively). The corn oil was used to simulate lipids and similar materials; the reference oil is a mixture of n-hexadecane, isooctane, and chlorobenzene. To sample 9 we added water (after spiking with the motor oil) to bring its water content to 30 percent. A total of 15 extractions were to be performed by each laboratory. The laboratories were instructed to extract three 3-g portions from each of matrices 1, 2, and 3, and all of the material they received for samples 4 through 9.

Sets of test samples were sent to 17 laboratories; 14 laboratories submitted extracts within the time frame specified by MRI-CO. The laboratories were to perform the extractions according to the instructions given in Appendix C and then ship the extracts to MRI-CO for IR analysis. To minimize errors due to reagent contamination, each laboratory was given tetrachloroethylene (PCE) for use as collection solvent, and anhydrous sodium sulfate that was to be added to the water-containing spiked clay sample. In addition, we made arrangements to provide each laboratory with the SFE-grade carbon dioxide from Scott Specialty Gases. However, due to delays in the arrival of the carbon dioxide, laboratories 05, 12, 15, and 17 used their own SFE-grade carbon dioxide (Air Products).

Apparatus

The SFE systems used by the laboratories in this study are identified in Table 2. The actual operating conditions, as reported by the laboratories, are given in Tables 3 through 8. The flow rates of the carbon dioxide reported by the participating laboratories are given in Table 9. The exact

TABLE 1. SAMPLES USED IN THE INTERLABORATORY STUDY[a]

Sample no.	Sample identification	TPH concentration (mg/kg)	Source
1A, B, C	TPH-1 soil (Lot 91017) without fatty acids	614[b]	Environmental Research Associates, Arvada, CO
2A, B, C	TPH-2 soil (Lot 91017) with fatty acids	2,050[b]	Environmental Research Associates, Arvada, CO
3A, B, C	SRS103-100 soil	32,600[c]	Fisher Scientific, Pittsburgh, PA
4	Clay soil spiked with motor oil	10,000[d]	MRI-CO[e]
5	Clay soil (unspiked)	--	MRI-CO
6	Clay soil spiked with motor oil	10,000[d]	MRI-CO
7	Clay soil spiked with motor oil	10,000[d]	MRI-CO
8	Clay soil spiked with corn oil and reference oil	1,000[d]	MRI-CO
9	Clay soil spiked with motor oil and water (approximately 30 percent by weight)	10,000[d]	MRI-CO

[a] Samples 1, 2, and 3 were extracted in triplicate by each laboratory. The three replicates were identified as A, B, and C. Only one extraction was performed for samples 4 through 9. Approximately 3 g of sample was extracted in each case. The exact amounts extracted by each laboratory are given in Table 10. The final volumes of the extracts before dilution are given in Table 11.

[b] Certified value.

[c] Determined by Soxhlet extraction with Freon-113 and analysis by IR spectrometry. Average of duplicate determinations.

[d] Spike value.

[e] Midwest Research Institute, California Operations.

TABLE 2. IDENTIFICATION OF THE SFE SYSTEMS USED IN THE COLLABORATIVE STUDY

Laboratory code	SFE system identification
01	Suprex SFE-50
02	Suprex Prepmaster
03	CCS Instruments SFE
04	Dionex-Lee Scientific SFE 703
05	Isco SFE System 1200
06	Suprex Prepmaster
08	Dionex-Lee Scientific SFE 703
10	HP 7680A
11	HP 7680A
12	HP 7680A
13	Isco SFE System 1200
14	Isco SFE System 1200
15	Isco SFE System 1200
17	Isco SFE System 1200

TABLE 3. SFE OPERATING CONDITIONS FOR THE ISCO SFE SYSTEMS

Parameter	05	13	14	15	17
CO_2 Pressure (atm)	340	340	340	340	340
CO_2 Density (g/mL)	0.785	0.785	0.785	0.785	0.785
CO_2 Flow rate (mL/min) (see Table 9)	Varied (see Table 9)	Varied (see Table 9)	Varied (see Table 9)	Varied (see Table 9)	Varied
Oven temperature (°C)	80	80	80	80	80
Extraction time (min)	30	30	30	30	30
Extraction vessel volume (mL)	10	2.5	2.5	10	2.5 and 10[a]
Extraction vessel dimensions 56-mm length	15-mm ID x 56-mm length	7.5-mm ID x 56-mm length	7.5-mm ID x 56-mm length	15-mm ID x 56-mm length	7.5/15-mm ID x
Extraction vessel orientation	Vertical, down flow	Vertical, down flow	Vertical, down flow	Vertical, down flow	Vertical, down flow
Restrictor dimensions 35-cm length	50-μm ID x 11-cm length	32-μm ID x 50-cm length	50-μm ID x 37.5-cm length	50-μm ID x 60/70-cm length[b]	50-μm ID x
Restrictor temperature (°C)	Not heated	80	Not heated	Not heated	Not heated
Collection solvent	PCE	PCE	PCE	PCE	PCE
Volume of solvent (mL)	3	3	3	3	3
Temperature of collection	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature[c]

[a] The 10-mL vessel was used for samples 3A, 3B, 3C, and 9.

[b] A 60-cm length restrictor was used with the 10-mL extraction vessel; a 70-cm length restrictor was used with the 2.5-mL extraction vessel.

[c] Initial temperature was room temperature; however, no attempt was made to control the collection solvent temperature during SFE.

TABLE 4. SFE OPERATING CONDITIONS FOR THE DIONEX-LEE SCIENTIFIC SFE-703 SYSTEMS

Parameter	04	08
CO_2 Pressure (atm)	340	340
CO_2 Density (g/mL)	0.785	0.785
CO_2 Flow rate (mL/min)	Varied (see Table 9)	Varied (see Table 9)
Oven temperature (°C)	80	80
Extraction time (min)	30	30
Extraction vessel volume (mL)	3.5	3.5
Extraction vessel dimensions	9.4-mm ID x 50-mm length	9.4-mm ID x 50-mm length
Extraction vessel orientation	Horizontal	Horizontal
Restrictor dimensions	a	a
Restrictor temperature (°C)	150	150
Collection solvent	PCE	PCE
Volume of solvent (mL)	5	5
Temperature of collection vial (°C)	2	2

[a] Restrictor identified as "250" restrictor.

TABLE 5. SFE OPERATING CONDITIONS FOR THE SUPREX PREPMASTER SFE SYSTEMS

Parameter	02	06
CO_2 Pressure (atm)	340	340
CO_2 Density (g/mL)	0.785	0.785
CO_2 Flow rate (mL/min)	1.2	Varied (see Table 9)
Oven temperature (°C)	80	80
Extraction time (min)	30	30
Extraction vessel volume (mL)	5	3
Extraction vessel dimensions	9.5-mm ID x 65-mm length	10-mm ID x 38-mm length
Extraction vessel orientation	Vertical, up flow	Vertical, up flow
Restrictor dimensions	a	50-μm ID x 10-cm length
Restrictor temperature (°C)	100	Not heated
Collection solvent	PCE	PCE
Volume of solvent (mL)	3	3
Temperature of collection vial (°C)	Ambient temperature[b]	Ambient temperature

[a] A prototype VariFlow restrictor (variable) was used.
[b] Initial temperature was ambient temperature; however, no attempt was made to control the collection solvent temperature during SFE.

TABLE 6. SFE OPERATING CONDITIONS FOR THE SUPREX SFE-50 SYSTEM

Parameter	01
CO_2 Pressure (atm)	340
CO_2 Density (g/mL)	0.785
CO_2 Flow rate (mL/min)	Varied (see Table 9)
Oven temperature (°C)	80
Extraction time (min)	30
Extraction vessel volume (mL)	3.5
Extraction vessel dimensions	9.4-mm ID x 50-mm length
Extraction vessel orientation	Horizontal
Restrictor dimensions	50-μm ID x 60-cm length
Restrictor temperature (°C)	Not heated
Collection solvent	PCE
Volume of solvent (mL)	3
Temperature of collection vial (°C)	Ambient temperature[a]

[a] Initial temperature was ambient temperature; however, no attempt was made to control the collection solvent temperature during SFE.

TABLE 7. SFE OPERATING CONDITIONS FOR THE CCS INSTRUMENTS SFE SYSTEM

Parameter	03
CO_2 Pressure (atm)	340
CO_2 Density (g/mL)	0.785
CO_2 Flow rate (mL/min)	0.2
Oven temperature (°C)	80
Extraction time (min)	30
Extraction vessel volume (mL)	6.0
Extraction vessel dimensions	9.5-mm ID x 127-mm length
Extraction vessel orientation	Vertical, up flow
Restrictor dimensions	20-μm ID x 5-cm length
Restrictor temperature (°C)	80 to 100
Collection solvent	PCE
Volume of solvent (mL)	3
Temperature of collection vial (°C)	Ambient temperature[a]

[a] Initial temperature was ambient temperature; however, no attempt was made to control the collection solvent temperature during SFE.

TABLE 8. SFE OPERATING CONDITIONS FOR THE HP 7680A SFE SYSTEMS

Parameter	10	11	12
CO_2 Pressure (atm)	340	370	370
CO_2 Density (g/mL)	0.78	0.80	0.80
CO_2 Flow rate (mL/min)	2.0	2.0	2.0
Oven temperature (°C)	80	80	80
Extraction time (min)	30	30	20
Extraction vessel volume (mL)	7.0	7.0	7.0
Extraction vessel dimensions	10-mm ID x 90-mm length	10-mm ID x 90-mm length	10-mm ID x 90-mm length
Volumes swept (mL)	10.2	9.9	6.6
Extraction vessel orientation	Vertical, up flow	Vertical, up flow	Vertical, up flow
Nozzle temperature (°C)	50	45	45
Trap temperature (°C)	-15	10	10
Trap packing material	Stainless-steel beads	Stainless-steel beads	ODS[a]
Rinse solvent	PCE	PCE	PCE
Volume	1.2	1.5	1.5
Rate (mL/min)	1.0	1.0	1.0
Nozzle temperature during rinse (°C)	40	45	45
Trap temperature during rinse (°C)	40	60	40
Number of rinses	2	2	2

[a] ODS - octadecylsilyl-bonded silica.

TABLE 9. FLOW RATE (mL/min) OF CARBON DIOXIDE (AS LIQUID) USED BY THE PARTICIPATING LABORATORIES

Sample ID	01	02	03	04	05	06	08	10	11	12	13	14[a]	15	17
1A	1.2	1.2	0.2	0.7	1.9	0.9	0.8	2.0	2.0	2.0	0.8		2.4	1.6
1B	1.2	1.2	0.2	0.7	1.7	0.7	0.7	2.0	2.0	2.0	0.8		2.3	1.6
1C	1.1	1.2	0.2	0.6	2.0	0.5	0.6	2.0	2.0	2.0	0.9		2.3	1.9
2A	1.0	1.2	0.2	0.7	1.9	1.2	0.7	2.0	2.0	2.0	0.6		2.2	1.6
2B	1.2	1.2	0.2	0.6	1.6	1.2	0.7	2.0	2.0	2.0	0.5		2.2	1.6
2C	1.0	1.2	0.2	0.6	1.9	0.6	0.6	2.0	2.0	2.0	0.6		2.0	1.2
3A	0.6	1.2	0.2	0.5	1.5	2.2	0.7	2.0	2.0	2.0	0.6		2.0	0.8
3B	0.5	1.2	0.2	0.6	1.5	1.2	0.6	2.0	2.0	2.0	0.6		2.0	0.8
3C	0.9	1.2	0.2	0.6	1.7	0.9	0.6	2.0	2.0	2.0	0.6		1.5	0.8
4	0.9	1.2	0.2	0.6	1.7	1.5	0.4	2.0	2.0	2.0	0.7		2.1	1.2
5	0.8	1.2	0.2	0.7	1.7	1.5	0.3	2.0	2.0	2.0	0.6		2.0	1.8
6	1.1	1.2	0.2	0.6	1.2	1.0	0.6	2.0	2.0	2.0	0.7		2.2	1.8
7	1.0	1.2	0.2	0.5	1.5	2.2	0.8	2.0	2.0	2.0	0.9		2.4	1.0
8	0.6	1.2	0.2	0.7	1.3	2.4	0.6	2.0	2.0	2.0	0.8		2.1	1.0
9	0.9	1.2	0.2	0.6	1.4	2.4	0.3	2.0	2.0	2.0	0.8		2.2	0.8

[a] Reported as 1 to 2 mL/min but not measured for each extraction.

weights of the samples extracted by the individual laboratories are listed in Table 10, and the volumes of the extracts delivered to MRI-CO are given in Table 11.

Two infrared spectrometers were used: a Perkin-Elmer Corporation (Norwalk, CT) Model 1605 FTIR, interfaced with a Digital Equipment Corporation (Cupertino, CA) DEC 386SX computer (scanning from 3200 to 2700 cm^{-1}), and a Buck-Scientific IR filter spectrometer Model 404, centered on 2924 cm^{-1} with a band of 15 cm^{-1} on each side (E. Norwalk, CT).

Materials

The reference oil standards used in the IR determination of TPHs were prepared as follows: Pipet 15 mL n-hexadecane, 15 mL isooctane, and 10 mL chlorobenzene into a 50-mL glass-stoppered flask. Mix by swirling the contents. Pipet 0.5 mL of this mixture into a tared 100-mL volumetric flask, weigh to the nearest milligram, and dilute to volume with PCE. Pipet appropriate volumes of this stock standard solution into 100-mL volumetric flasks and dilute to volume with PCE to make 10-μg/mL, 25-μg/mL, 50-μg/mL, 100-μg/mL, 250-μg/mL, and 500-μg/mL calibration standards. The motor oil calibration standards at 10 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, 250 μg/mL, and 500 μg/mL were prepared by serial dilution of a stock solution made at 200 μg/μL in PCE. Corn oil at 40 mg/mL in Freon-113 was used to spike the clay soil.

The following materials were used in the study:

n-Hexadecane, isooctane, chlorobenzene (Aldrich Chemical, Milwaukee, WI).

Corn oil (local supermarket).

Motor oil (local Shell gas station).

PCE, glass-distilled, HPLC grade, lot no. 06626TX (Aldrich Chemical).

Silica gel, 70 to 230 mesh, ASTM (Baxter Scientific Products, McGaw Park, IL).

Anhydrous sodium sulfate, analytical reagent (Mallinckrodt, St. Louis, MO).

Anhydrous magnesium sulfate, analytical reagent (Mallinckrodt).

Hydromatrix (diatomaceous earth) (Isco, Inc., Lincoln, NB).

Clay soil (Sandoz Experimental Station, San Jose, CA) (33.6% sand, 35.4% silt, 31% clay; organic carbon 1.8%, water 10.6%).

Carbon dioxide, SFC/SFE-grade (Air Products, Allentown, PA).

Carbon dioxide, SFE-grade (Scott Specialty Gases, Inc., Plumsteadville, PA).

The standard reference materials used are listed in Table 1.

TABLE 10. WEIGHTS (g) OF THE SAMPLES EXTRACTED IN THE STUDY

Laboratory code	1A	1B	1C	2A	2B	2C	3A	3B	3C	4	5	6	7	8	9
01	3.03	3.00	3.03	3.00	3.03	3.04	2.98	3.07	3.05	3.02	3.00	3.00	3.02	3.01	1.62
02	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	2.40
03	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	a	3.00	3.00	3.00	3.00	3.00	3.00
04	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.02	2.98	3.00	3.01	2.99	4.29
05	3.03	3.00	3.01	3.00	3.01	3.01	3.06	3.01	3.05	3.01	2.99	3.01	3.01	3.00	3.01
06	3.00	3.00	3.00	3.01	3.00	3.01	3.00	3.00	3.00	3.00	2.99	3.00	3.00	3.07	2.91
08	3.09	3.02	3.03	3.02	3.01	3.07	3.11	2.98	3.02	3.01	3.00	2.99	3.00	2.99	2.78
10	3.12	3.07	2.99	3.01	3.00	3.00	3.02	3.00	3.03	2.99	2.99	2.98	3.00	2.99	2.52
11	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	2.99	3.00	3.00	3.01	3.03	2.34
12	3.09	3.07	3.10	3.03	3.10	3.06	3.03	3.10	b	3.01	2.98	3.00	3.00	2.99	2.48
13	3.01	2.98	3.00	3.01	3.00	3.00	2.99	3.03	3.07	3.01	2.99	3.01	3.02	3.00	2.86
14	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	1.24	3.00	3.00	2.70	3.00	2.50	3.00
15	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	2.55
17	3.00	3.00	3.00	3.01	3.00	3.00	3.02	3.03	3.01	3.00	2.99	3.00	3.00	3.04	2.95

[a] The extraction was not performed.

[b] The extract was not submitted because the SFE system developed a leak.

TABLE 11. FINAL VOLUMES OF THE EXTRACTS (mL) BEFORE DILUTION

Laboratory code	1A	1B	1C	2A	2B	2C	3A	3B	3C	4	5	6	7	8	9
01	3.8	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
02	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
03	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	a	3.0	3.0	3.0	3.0	3.0	3.1
04	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.6	4.6	4.5	4.4	4.5	4.6	4.5	4.4
05	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.1	3.0	3.0	3.0	3.4	3.0
06	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
08	4.6	4.7	4.7	4.7	4.7	4.7	4.8	4.7	4.7	4.6	a	4.6	4.6	4.6	4.8
10	3.0	3.0	3.0	3.0	3.0	3.0	3.9	3.8	3.0	3.0	3.0	3.2	3.1	3.0	3.0
11	3.4	3.4	3.4	3.3	3.3	3.4	3.6	3.6	3.6	3.5	3.0	3.5	3.5	3.3	3.4
12	3.2	3.2	3.2	3.2	3.2	3.2	3.3	3.4	a	3.3	3.3	3.3	3.4	3.3	3.4
13	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
14	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
15	3.0	3.0	3.0	3.3	3.0	3.2	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
17	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

[a] The extract was not submitted for analysis.

Sample Spiking Procedure

For spiking of the clay soil samples with motor oil, 3.0-g portions of the soil were weighed into 7-mL glass vials, and portions (150 μL) of concentrated stock solution containing motor oil in Freon-113 at 200 μg/μL were added with a syringe while ensuring that the solution did not contact the walls of the vials. Mixing was performed with the tip of a disposable glass pipet. After the solvent had completely evaporated (approximately 15 min), the vials with the spiked samples were sealed with Teflon-lined caps and stored at 4°C for 5 days prior to shipping to the laboratories. Spiking with corn oil and reference oil was done as described above for the motor oil, except that the concentrations of the stock solutions were 20 μg/μL. The clay soil samples that required adjustment of their water content to 30 percent were also spiked individually; a 2.4-g portion of the clay soil sample was weighed into a glass vial, spiked, and then 0.6 mL water was added.

Sample Extraction

The methods used to extract the samples and to analyze the extracts are included in Appendices A and B to this report. Each laboratory used only one analyst to perform sample extractions and allowed one block of time for all sample extractions.

TREATMENT OF DATA

Outlier Testing

Outlier testing was done using both the Cochran test and the Grubbs test (3, 4). The former is used for the removal of results from laboratories that show significantly greater variability among replicate (intralaboratory) analyses than the other laboratories for a given material. To calculate the Cochran test statistic, the intralaboratory variance for each laboratory was computed, and the largest of these values was divided by the sum of all these variances. When a laboratory was rejected on the basis of these tests, its results were removed from the set of data for a particular matrix, and the test was repeated using the remaining data in the subset.

Grubbs tests were performed to remove results from laboratories with extreme averages. The single-value test (2-tail; $P = 0.01$) was run first; then, when no outlier was found, the pair value test (two values at the highest end, two values at the lowest end, and two values, one at each end, at an overall $P = 0.01$) was applied.

To calculate the single Grubbs test statistic, the average for each of the L laboratories was computed, and the standard deviation of these L averages (designated as the original s) was calculated. The standard deviation of the set of averages with the highest average removed (s_H) was calculated, then the standard deviation of the set of averages with the lowest average removed (s_L) was calculated. Finally, the percentage decrease in standard deviation was calculated as follows:

$$100 \times [1 - (s_L/s)] \text{ and } 100 \times [1-(s_H/s)]$$

The higher of these two percentage decreases was the single Grubbs statistic, which signals the presence of an outlier to be omitted if it *exceeds* the critical value listed in the single Grubbs tables, at the $P = 0.01$ level, 2-tail, for L laboratories.

To calculate the Grubbs pair statistic, we proceeded in an analogous fashion, except that we calculated the standard deviations, from the original set of averages. The smallest of these three standard deviation values was taken and the corresponding percentage in standard deviation from the original s was calculated. A Grubbs outlier pair was present if the selected value for the percentage decrease from the original s *exceeded* the critical value listed in the Grubbs pair value table, at the $P = 0.01$ level, for L laboratories.

Statistical Analysis

Statistical analysis was performed using the AOAC Lotus spreadsheet program developed for the analysis of data from interlaboratory studies (4).

The summary statistics that are reported for each matrix include the mean concentration of TPHs and the method precision (percent relative standard deviation). The repeatability relative standard deviation (RSD_r), which was determined from the repeatability standard deviation (s_r) and the mean concentration of a particular matrix, is an indication of the intralaboratory precision. The reproducibility relative standard deviation (RSD_R), which was determined from the reproducibility standard deviation (s_R) and the mean concentration of a particular matrix, is an indication of the interlaboratory method precision.

QUALITY ASSURANCE/QUALITY CONTROL

The extractions of the study samples were performed according to the instructions provided by MRI-CO to all collaborators (Appendix C). To minimize errors due to reagent contamination, we provided each laboratory with the collection solvent, the extractant (carbon dioxide), and the drying agent (anhydrous sodium sulfate). We made arrangements with Scott Specialty Gases to supply carbon dioxide from the same lot to all participating laboratories. All but four laboratories used SFE-grade carbon dioxide from Scott Specialty Gases to extract the test samples. Laboratories 05,12,15, and 17 experienced delay in delivery of the carbon dioxide and therefore used SFC/SFE-grade carbon dioxide from Air Products. Each laboratory was instructed to report the SFE operating conditions on special forms (provided by MRI-CO), and to record the exact mass of the study sample extracted by SFE.

The IR analyses were performed on two different IR systems according to the proposed Method 8440 included in Appendix B. The following quality control procedures were implemented:

- A six-level calibration (at 10, 25, 50, 100, 250, and 500 μg/mL) was performed every day, on each instrument, during the period the IR analyses were performed. The multilevel calibration was then verified after every 10 analyses by analyzing a reference oil standard or a motor oil standard at 100 μg/mL. The reference oil standard was used to quantify TPHs in samples 1, 2, 3, 5, and 8. The motor oil standard was used to quantify TPHs in samples 4, 6, 7, and 9. Corn oil was quantified against the reference oil standard.

- PCE blanks were analyzed daily on each instrument during the period the IR analyses were performed.

- All system blanks received from the participating laboratories were analyzed for TPHs.

- Sample 5 was a blind QC sample (unspiked clay soil).

At the MRI-CO, six clay soil samples were spiked with motor oil at 10,000 mg/kg and stored at 4°C in the dark; two were extracted with supercritical carbon dioxide after 22 days, two after 34 days, and two after 40 days of storage. These six samples were part of the batch of samples prepared for the collaborative study and were expressly set aside for extraction at a later time but still within the time frame in which the collaborating laboratories would carry out their extractions (six laboratories submitted extracts within 30 days of sample spiking, five within 40 days, and three within 60 days).

SECTION 5

RESULTS AND DISCUSSION

INTERLABORATORY METHOD PERFORMANCE

The results of the interlaboratory study have been summarized by matrix and by the IR spectrometer used in the analysis. As mentioned in Section 1, all IR determinations were performed at MRI-CO on two different IR spectrometers. Tables 12 through 19 present the concentration data for each of the four matrices that were extracted in triplicate (TPH-1 soil, TPH-2 soil, SRS103-100 soil, and the clay soil spiked with motor oil). The mean concentrations, the repeatability standard deviations (s_r), the reproducibility standard deviations (s_R), the repeatability relative standard deviations (RSD_r), the reproducibility relative standard deviations (RSD_R) and the mean recoveries have been summarized in Tables 20 through 25.

Rejection of Outliers

For the entire study, the AOAC software program rejected only one laboratory (laboratory 03) on two matrices. This laboratory achieved very low recoveries on all test samples because the carbon dioxide flow rate was too low (0.2 mL/min); however, the outlier test only rejected values on the TPH-2 soil extract and on the extract from the clay soil spiked with motor oil. Table 26 summarizes the outlier testing results. When data from all laboratories were pooled, and the outlier testing was performed by matrix, then laboratory 03 data for TPH-2 and spiked clay soil samples were rejected on the basis of the single Grubbs test (lowest average). When data from five laboratories using Isco systems were pooled, and outlier testing was performed by matrix, then laboratory 14 data were rejected on the basis of the Cochran test (maximum intralaboratory variance). When data from laboratories using extraction vessels of 3.5 mL or less in volume were pooled, and outlier testing was performed by matrix, then data from laboratory 17 were rejected on the basis of the Cochran test (maximum intralaboratory variance).

Method Recovery

The summary statistics for the recoveries from each of the four matrices that were extracted by all laboratories, calculated after outlier removal, are presented in Table 21. The mean recoveries of TPHs from these four matrices ranged from 77.9 to 107 percent for analyses performed with the PE-FTIR spectrometer, and from 75.9 to 101 percent for analyses performed with the BSci-IR spectrometer (a discussion of the correlation between the data generated with the two IR spectrometers is given later in this section). These recoveries are in agreement with data that we reported previously for the single-laboratory evaluation of this method (1, 2); the percent differences between the mean recoveries for the interlaboratory study and the single-laboratory study were ranging from 1.8 to 28.7 percent.

TABLE 12. CONCENTRATIONS (mg/kg) OF TPHs DETERMINED IN TPH-1 SOIL EXTRACTS USING THE PE-FTIR SPECTROMETER[a]

Laboratory code	Concentration (mg/kg)				Percent RSD
	X_1	X_2	X_3	Mean	
01	618	808	1,000	809	23.6
02	449	453	487	463	4.5
03	67	ND[b]	77	51.3	70.5
04	352	391	420	388	8.8
05	943	859	854	885	5.7
06	250	478	44	257	84.5
08	737	799	933	823	12.2
10	693	1,070	789	851	23.0
11	972	1,210	1,100	1,090	10.9
12	691	701	656	683	23.6
13	673	604	620	632	5.7
14	639	605	580	608	4.9
15	883	963	885	910	5.0
17	575	712	832	706	18.2

[a] The certified concentration of TPHs in this sample is 614 mg/kg.
[b] ND - not detected; the estimated detection limit is 10 mg/kg.

TABLE 13. CONCENTRATIONS (mg/kg) OF TPHs DETERMINED IN TPH-1 SOIL EXTRACTS USING THE BSci-IR SPECTROMETER[a]

Laboratory code	Concentration (mg/kg)				Percent RSD
	X_1	X_2	X_3	Mean	
01	634	793	987	805	22.0
02	402	393	433	409	5.1
03	62.0	ND[b]	80.5	50.8	72.0
04	339	386	404	376	8.9
05	922	831	825	859	6.3
06	230	435	52.5	239	80.0
08	751	821	940	837	11.4
10	590	1,060	690	780	31.7
11	959	1,190	1,080	1,080	10.7
12	621	625	593	613	2.8
13	594	535	544	558	5.7
14	563	532	510	535	5.0
15	859	944	856	886	5.6
17	497	639	747	628	20.0

[a] The certified concentration of TPHs in this sample is 614 mg/kg.
[b] ND - not detected; the estimated detection limit is 10 mg/kg.

TABLE 14. CONCENTRATIONS (mg/kg) OF TPHs DETERMINED IN TPH-2 SOIL EXTRACTS USING THE PE-FTIR SPECTROMETER[a]

Laboratory code	Concentration (mg/kg)				Percent RSD
	X_1	X_2	X_3	Mean	
01	1,840	2,160	1,880	1,960	8.9
02	1,940	1,990	1,730	1,890	7.3
03	257	410	356	341	22.8
04	1,350	1,160	1,520	1,340	13.4
05	1,490	2,000	2,230	1,910	19.8
06	1,850	1,730	1,380	1,650	14.8
08	1,480	1,100	1,500	1,360	16.7
10	1,880	2,240	1,980	2,030	9.2
11	2,140	2,200	2,050	2,130	3.5
12	2,320	2,440	2,110	2,290	7.3
13	1,900	2,010	1,940	1,950	2.9
14	2,130	1,900	1,340	1,790	22.7
15	1,850	1,900	1,990	1,910	3.7
17	1,780	1,920	1,980	1,890	5.4

[a] The certified concentration of TPHs in this sample is 2,050 mg/kg.

TABLE 15. CONCENTRATIONS (mg/kg) OF TPHs DETERMINED IN TPH-2 SOIL EXTRACTS USING THE BSci-IR SPECTROMETER[a]

Laboratory code	Concentration (mg/kg)				Percent RSD
	X_1	X_2	X_3	Mean	
01	1,620	1,950	1,660	1,740	10.4
02	1,760	1,790	1,540	1,700	8.0
03	250	374	380	335	21.9
04	1,290	1,120	1,460	1,290	13.2
05	1,370	1,740	1,980	1,700	18.1
06	1,670	1,550	1,240	1,490	14.9
08	1,350	1,050	1,330	1,240	13.5
10	1,660	2,010	1,770	1,810	9.9
11	1,960	2,040	1,910	1,970	3.3
12	2,110	2,210	1,780	2,030	11.1
13	1,670	1,760	1,710	1,710	2.6
14	1,900	1,680	1,180	1,590	23.2
15	1,700	1,730	1,820	1,750	3.6
17	1,530	1,680	1,730	1,650	6.3

[a] The certified concentration of TPHs in this sample is 2,050 mg/kg.

TABLE 16. CONCENTRATIONS (mg/kg) OF TPHs DETERMINED IN SRS103-100 SOIL EXTRACTS USING THE PE-FTIR SPECTROMETER[a]

Laboratory code	Concentration (mg/kg)				Percent RSD
	X_1	X_2	X_3	Mean	
01	32,600	35,600	33,700	34,000	4.5
02	28,900	29,600	29,500	29,300	12.9
03	4,130	11,100	b	7,620	64.7
04	23,200	20,800	25,000	23,000	9.2
05	25,200	29,500	10,800	21,800	44.9
06	46,400	45,100	54,600	48,700	10.6
08	25,500	24,400	24,400	24,800	2.6
10	19,500	14,800	17,500	17,300	13.6
11	28,900	27,800	28,300	28,300	1.9
12	33,200	32,700	b	32,900	1.1
13	30,900	30,900	31,500	31,100	1.1
14	26,500	29,200	11,400	22,400	42.8
15	31,100	32,200	30,800	31,400	2.3
17	21,600	24,300	22,600	22,800	6.0

[a] The concentration of TPHs measured in our laboratory for this sample is 32,600 mg/kg.
[b] This laboratory did not submit an extract for this sample.

TABLE 17. CONCENTRATIONS (mg/kg) OF TPHs DETERMINED IN SRS103-100 SOIL EXTRACTS USING THE BSci-IR SPECTROMETER[a]

Laboratory code	Concentration (mg/kg)				Percent RSD
	X_1	X_2	X_3	Mean	
01	29,600	32,200	30,500	30,800	4.3
02	27,000	27,800	27,900	27,600	1.8
03	3,890	10,200	b	7,050	63.3
04	22,700	20,600	24,400	22,600	8.4
05	22,400	25,800	9,790	19,300	43.7
06	42,400	41,100	49,100	44,200	9.7
08	23,600	23,200	23,800	23,500	1.3
10	18,300	14,000	16,200	16,200	13.3
11	26,600	25,100	25,700	25,800	2.9
12	30,500	30,300	b	30,400	0.5
13	27,900	27,600	28,600	28,000	1.8
14	24,500	26,800	11,300	20,900	40.0
15	28,300	29,400	28,200	28,600	2.3
17	20,600	22,800	21,300	21,600	5.2

[a] The concentration of TPHs measured in our laboratory for this sample is 32,600 mg/kg.
[b] This laboratory did not submit an extract for this sample.

TABLE 18. CONCENTRATIONS (mg/kg) OF TPHs DETERMINED IN SPIKED CLAY SOIL EXTRACTS USING THE PE-FTIR SPECTROMETER[a]

Laboratory code	Concentration (mg/kg)				Percent RSD
	X_1	X_2	X_3	Mean	
01	8,760	10,400	8,680	9,280	10.5
02	7,850	8,740	9,740	8,780	10.8
03	580	368	963	637	47.3
04	7,000	7,590	7,270	7,290	4.1
05	8,160	8,790	9,290	8,750	6.5
06	6,240	6,810	4,100	5,720	25.0
08	7,110	6,130	5,780	6,340	10.9
10	6,470	4,310	3,200	4,660	35.7
11	9,690	8,380	9,400	9,160	7.5
12	8,660	8,810	9,270	8,910	3.6
13	8,420	8,210	8,830	8,490	3.7
14	5,000	7,910	8,970	7,290	28.2
15	8,560	7,660	9,020	8,410	8.2
17	7,990	7,940	8,590	8,170	4.4

[a] The clay soil samples were spiked with motor oil at 10,000 mg/kg.

TABLE 19. CONCENTRATIONS (mg/kg) OF TPHs DETERMINED IN SPIKED CLAY SOIL EXTRACTS USING THE BSci-IR SPECTROMETER[a]

Laboratory code	Concentration (mg/kg)				Percent RSD
	X_1	X_2	X_3	Mean	
01	9,220	10,800	8,870	9,630	10.7
02	8,020	9,380	9,980	9,130	11.0
03	586	411	954	650	42.6
04	7,180	7,670	7,270	7,370	3.5
05	8,440	9,120	9,480	9,010	5.9
06	6,040	6,610	6,760	6,470	5.9
08	7,460	6,650	6,150	6,750	9.8
10	7,310	5,230	4,130	5,560	29.0
11	9,880	9,000	9,770	9,550	5.0
12	9,110	9,210	9,310	9,210	1.1
13	8,720	8,670	9,020	8,800	2.2
14	5,400	8,360	9,220	7,660	26.2
15	8,770	8,120	9,680	8,860	8.8
17	7,970	8,120	8,920	8,340	6.1

[a] The clay soil samples were spiked with motor oil at 10,000 mg/kg.

TABLE 20. SUMMARY OF RESULTS FROM ALL LABORATORIES (BEFORE OUTLIER REMOVAL)

Matrix	Certified or spike value (mg/kg)	Mean concentration[a] (mg/kg)	s_r (mg/kg)	s_R (mg/kg)	Percent RSD_r	Percent RSD_R	Percent mean recovery	Number of laboratories in the study
PE-FTIR								
ERA TPH-1 soil	614	654	111	297	17.0	45.4	107	14
ERA TPH-2 soil	2,050	1,750	206	509	11.8	29.2	85.4	14
SRS103-100 soil	32,600	26,820	4,320	9,720	16.1	36.2	82.3	14
Clay soil spiked with motor oil	10,000	7,280	968	2,490	13.3	34.2	72.8	14
BSci-IR								
ERA TPH-1 soil	614	618	113	296	18.2	47.9	101	14
ERA TPH-2 soil	2,050	1,570	188	446	12.0	28.4	76.7	14
SRS103-100 soil	32,600	24,750	3,740	8,650	15.1	35.0	75.9	14
Clay soil spiked with motor oil	10,000	7,640	88	2,470	11.5	32.4	76.4	14

[a] The number of replicates per laboratory was three.

TABLE 21. SUMMARY OF RESULTS FROM ALL LABORATORIES (AFTER OUTLIER REMOVAL)

Matrix	Certified or spike value (mg/kg)	Mean concentration[a] (mg/kg)	s_r (mg/kg)	s_R (mg/kg)	Percent RSD_r	Percent RSD_R	Percent mean recovery	Number of laboratories retained
PE-FTIR								
ERA TPH-1 soil	614	654	111	297	17.0	45.4	107	14
ERA TPH-2 soil	2,050	1,850	213	321	11.5	17.3	90.2	13
SRS103-100 soil	32,600	26,820	4,320	9,720	16.1	36.2	82.3	14
Clay soil spiked with motor oil	10,000	7,790	1,000	1,660	12.9	21.3	77.9	13
BSci-IR								
ERA TPH-1 soil	614	618	113	296	18.2	47.9	101	14
ERA TPH-2 soil	2,050	1,670	194	278	11.7	16.7	81.5	13
SRS103-100 soil	32,600	24,750	3,740	8,650	15.1	35.0	75.9	14
Clay soil spiked with motor oil	10,000	8,180	910	1,500	11.1	18.3	81.8	13

[a] The number of replicates per laboratory was three.

TABLE 22. SUMMARY OF RESULTS FROM LABORATORIES USING ISCO SYSTEMS (BEFORE OUTLIER REMOVAL)

Matrix	Certified or spike value (mg/kg)	Mean concentration[a] (mg/kg)	s_r (mg/kg)	s_R (mg/kg)	Percent RSD_r	Percent RSD_R	Percent mean recovery	Number of laboratories retained
PE-FTIR								
ERA TPH-1 soil	614	748	68.3	152	9.1	20.3	122	5
ERA TPH-2 soil	2,050	1,890	256	256	13.5	13.5	92.2	5
SRS103-100 soil	32,600	25,900	6,170	7,020	23.8	27.1	79.4	5
Clay soil spiked with motor oil	10,000	8,220	1,030	1,030	12.5	12.5	82.2	5
BSci-IR								
ERA TPH-1 soil	614	693	67.7	177	9.8	25.5	113	5
ERA TPH-2 soil	2,050	1,680	222	222	13.3	13.3	82.0	5
SRS103-100 soil	32,600	23,700	5,350	6,150	22.6	26.0	72.7	5
Clay soil spiked with motor oil	10,000	8,530	1,020	1,020	12.0	12.0	85.3	5

[a] The number of replicates per laboratory was three.

TABLE 23. SUMMARY OF RESULTS FROM LABORATORIES USING ISCO SYSTEMS (AFTER OUTLIER REMOVAL)

Matrix	Certified or spike value (mg/kg)	Mean concentration[a] (mg/kg)	s_r (mg/kg)	s_R (mg/kg)	Percent RSD_r	Percent RSD_R	Percent mean recovery	Number of laboratories retained
PE-FTIR								
ERA TPH-1 soil	614	748	68.3	152	9.1	20.3	122	5
ERA TPH-2 soil	2,050	1,890	256	256	13.5	13.5	92.2	5
SRS103-100 soil	32,600	25,900	6,170	7,020	23.8	27.1	79.4	5
Clay soil spiked with motor oil	10,000	8,460	507	507	6.0	6.0	84.6	4
BSci-IR								
ERA TPH-1 soil	614	693	67.7	177	9.8	25.5	113	5
ERA TPH-2 soil	2,050	1,680	222	222	13.3	13.3	82.0	5
SRS103-100 soil	32,600	23,700	5,350	6,150	22.6	26.0	72.7	5
Clay soil spiked with motor oil	10,000	8,530	1,020	1,020	12.0	12.0	85.3	5

[a] The number of replicates per laboratory was three.

TABLE 24. SUMMARY OF RESULTS FROM LABORATORIES USING EXTRACTION VESSELS 3.5 mL OR LESS IN VOLUME (BEFORE OUTLIER REMOVAL)

Matrix	Certified or spike value (mg/kg)	Mean concentration[a] (mg/kg)	s_r (mg/kg)	s_R (mg/kg)	Percent RSD_r	Percent RSD_R	Percent mean recovery	Number of laboratories retained
PE-FTIR								
ERA TPH-1 soil	614	603	127	236	21.1	39.1	98.2	7
ERA TPH-2 soil	2,050	1,710	224	322	13.1	18.9	83.4	7
SRS103-100 soil	32,600	30,650	4,580	10,660	14.9	34.8	94.0	6
Clay soil spiked with motor oil	10,000	7,510	1,070	1,520	14.2	20.2	75.1	7
BSci-IR								
ERA TPH-1 soil	614	568	117	236	20.6	41.4	92.5	7
ERA TPH-2 soil	2,050	1,530	203	259	13.3	16.9	74.6	7
SRS103-100 soil	32,600	28,300	3,960	9,190	14.0	32.4	86.8	6
Clay soil spiked with motor oil	10,000	7,860	927	1,360	11.8	17.3	78.6	7

[a] The number of replicates per laboratory was three.

TABLE 25. SUMMARY OF RESULTS FROM LABORATORIES USING EXTRACTION VESSELS 3.5 mL OR LESS IN VOLUME (AFTER OUTLIER REMOVAL)

Matrix	Certified or spike value (mg/kg)	Mean concentration[a] (mg/kg)	s_r (mg/kg)	s_R (mg/kg)	Percent RSD_r	Percent RSD_R	Percent mean recovery	Number of laboratories retained
PE-FTIR								
ERA TPH-1 soil	614	603	127	236	21.1	39.1	98.2	7
ERA TPH-2 soil	2,050	1,710	224	322	13.1	18.9	83.4	7
SRS103-100 soil	32,600	32,300	2,600	10,420	8.1	32.3	99.1	5
Clay soil spiked with motor oil	10,000	7,510	1,070	1,520	14.2	20.2	75.1	7
BSci-IR								
ERA TPH-1 soil	614	568	117	236	20.6	41.4	92.5	7
ERA TPH-2 soil	2,050	1,530	203	259	13.3	16.9	74.6	7
SRS103-100 soil	32,600	29,820	2,200	8,890	7.4	29.8	91.5	5
Clay soil spiked with motor oil	10,000	7,860	927	1,360	11.8	17.3	78.6	7

[a] The number of replicates per laboratory was three.

TABLE 26. OUTLIER TESTING—THE COCHRAN STATISTIC, THE SINGLE GRUBBS STATISTIC, AND THE DOUBLE GRUBBS STATISTIC[a]

Instrument[b]	Matrix	Number of laboratories	Cochran test	Single Grubbs test	Double Grubbs test
All laboratories					
1	TPH-1 soil	14	0.2725	17.86	29.75
2	TPH-1 soil	14	0.3448	15.27	25.12
1	TPH-2 soil	14	0.2781	43.77[c]	51.67[c]
		13	0.2810	14.06	38.22
2	TPH-2 soil	14	0.2739	45.42[c]	52.68[c]
		13	0.2769	13.30	31.38
1	SRS103-100 soil	14	0.3768	22.58	44.00
2	SRS103-100 soil	14	0.3699	22.01	45.07
1	Clay soil spiked with motor oil	14	0.3219	38.91[c]	51.60[c]
		13	0.3242	20.78	36.35
2	Clay soil spiked with motor oil	14	0.3703	45.13[c]	54.43[c]
		13	0.3729	16.96	28.01
Laboratories using Isco SFE system					
1	TPH-1 soil	5	0.7097	11.29	63.77
2	TPH-1 soil	5	0.6868	11.62	71.21
1	TPH-2 soil	5	0.5046	59.63	83.00
2	TPH-2 soil	5	0.5504	32.38	57.00
1	STS103-100 soil	5	0.5036	9.95	89.75
2	SRS103-100 soil	5	0.4973	11.24	73.54
1	Clay soil spiked with motor oil	5	0.8041[c]	57.72	70.64
		4	0.4649	30.57	78.03
2	Clay soil spiked with motor oil	5	0.7715	47.04	80.15
Laboratories using extraction vessels 3.5 mL or less in volume					
1	TPH-1 soil	7	0.4151	24.18	53.41
2	TPH-1 soil	7	0.3833	19.07	34.84
1	TPH-2 soil	7	0.4690	12.88	51.37
2	TPH-2 soil	7	0.4732	15.40	48.30
1	SRS103-100 soil	6	0.7314[c]	48.02	60.03
		5	0.7850	49.23	58.26
2	SRS103-100 soil	6	0.7434[c]	52.24	64.41
		5	0.7630	55.74	66.48
1	Clay soil spiked with motor oil	7	0.5280	15.71	31.75
2	Clay soil spiked with motor oil	7	0.6672	20.64	34.53

[a] The critical values of the Cochran statistic, the single Grubbs statistic, and the double Grubbs statistic are given in Reference 3.
[b] Instrument 1 is the PE-FTIR spectrometer; instrument 2 is the BSci-IR spectrometer.
[c] This value is an outlier.

We pooled all data generated with the five Isco SFE systems used in this study. Tables 22 and 23 present the summary statistics for these data before and after outlier removal. The method recoveries ranged from 79.4 to 122 percent for PE-FTIR analyses and from 72.7 to 113 percent for BSci-IR analyses. When the results from all laboratories using extraction vessels with a volume of 3.5 mL or less were pooled (seven laboratories), the mean recoveries ranged from 75.1 to 99.1 percent for PE-FTIR analyses and from 74.6 to 92.5 percent for BSci-IR analyses (Tables 24 and 25).

We performed a 2-sample t-test to determine whether the data in Tables 21, 23, and 25 (specifically, percent recoveries for each of the four matrices) are different from each other at the 5-percent significance level. When comparing the data in Tables 21 and 23, we find significant differences only for the clay soil matrix; when comparing the data in Tables 21 and 25, we find significant differences only for the SRS103-100 soil matrix; and finally, when comparing the data in Tables 23 and 25, we find significant differences for all matrices.

To determine the overall method recovery, we performed a linear regression of the data presented in Tables 12, 14, 16, and 18 (after removal of outliers). The measured concentrations for the PE-FTIR analyses were plotted on the y-axis, and the true concentrations of TPHs in the four matrices were plotted on the x-axis. The slope of the regression equation was 0.8291, and the intercept was -18.87. The correlation coefficient was 0.9121 (160 data points). These data indicate that the overall method recovery (for levels ranging from 614 mg/kg to 32,600 mg/kg) is 82.9 percent. Since the true concentrations for the three reference materials were obtained by extracting them with Freon-113 and analyzing the extract by IR, and the fourth true value was the spike concentration, we concluded that the performance of Method 3560/8440 (SFE/IR) was comparable to that of Method 9071A/8440 (Freon-113 extraction/IR analysis).

Method Precision

The interlaboratory standard deviation (s_R, reproducibility) is the precision associated with measurements generated by a group of laboratories; the single-analyst standard deviation (s_r, repeatability) is the precision associated with performance in an individual laboratory. The values for s_r and s_R are given in Tables 20 through 25; they were used to calculate the method precision, given as the repeatability relative standard deviation (RSD_r) and the reproducibility relative standard deviation (RSD_R).

When data from all laboratories were pooled and outliers removed, the RSD_r ranged from 11.5 to 17.0 percent for PE-FTIR analyses and from 11.1 to 18.2 percent for BSci-IR analyses. The RSD_R ranged from 17.3 to 45.4 percent for PE-FTIR analyses and from 16.7 to 47.9 percent for BSci-IR analyses.

When the data from the Isco SFE systems were pooled and outliers removed, the percent RSD_r ranged from 6.0 to 23.8 percent for the PE-FTIR analyses and from 9.8 to 22.6 percent for BSci-IR analyses. The RSD_R ranged from 6.0 to 27.1 percent for PE-FTIR analyses and from 12 to 26 percent for BSci-IR analyses. The interlaboratory method precision for the Isco SFE systems alone (RSD_R in Table 23) was better than that for all SFE systems (RSD_R in Table 25), because the Isco RSD_R values were lower for each of the four matrices.

The method precision for the seven laboratories using extraction vessels with a volume of 3.5 mL or less was almost similar to that achieved for all laboratories. For example, the RSD_r in Table 25 ranged from 8.1 to 21.1 percent for PE-FTIR analyses and from 7.4 to 20.6 percent for BSci-IR analyses, and the RSD_R ranged from 18.9 to 39.1 percent for PE-FTIR analyses and from 16.9 to 41.4 percent for BSci-IR analyses. This seems to indicate that the vessel size within the range used in this study was of little importance.

The precision estimates for the SFE/IR method are ± 20 percent for the intralaboratory performance and ± 45 percent for the interlaboratory performance.

Analysis of Variance

Analysis of variance (ANOVA) was used to separate (mathematically) the total variation of the experimental measurements into an intralaboratory portion and an interlaboratory portion, with a corresponding split of the total number of degrees of freedom. The mean squares for the among laboratories and within laboratories were calculated from the sum of squares and the number of degrees of freedom. The ratio of the two mean squares (the mean of squares among laboratories and the mean of squares within laboratories) is distributed as F. Thus, we calculated the F and compared it with the critical values of F for the upper 95 percent point of distribution at the corresponding degrees of freedom. For example, in Table 27, we are performing an ANOVA for data submitted by the 14 laboratories for the TPH-1 sample; in each case, we have three replicates per laboratory. The among-laboratories degrees of freedom is the number of laboratories (14 minus 1). The within-laboratories degrees of freedom is the number of laboratories multiplied by 2; the number 2 in this case is the number of replicates minus 1. The ratio of the mean squares is 19.4. Since the critical value of F is 2.09, we see that the variation from laboratory to laboratory was greater than that attributed to the analytical error displayed within laboratories. The matrix and operational parameters such as flow rate, extraction vessel design and orientation, mode of collection of the extracted material, and temperature of the collection solvent/trap seemed to be important.

The ANOVA results for the other three matrices are presented in Tables 28 through 30. In all cases, we find that the interlaboratory variance is greater than intralaboratory variance for the reasons stated above.

To further substantiate that the interlaboratory variance can be attributed to matrix, we pooled the data obtained with the Isco SFE systems (laboratories 05, 13, 14, 15, and 17). In this case, all laboratories used an extraction vessel in vertical position, and the collection vial was kept in a beaker with water at room temperature. The vessel dimensions, however, varied slightly, the restrictor dimensions varied slightly, and the restrictor temperature was different for one laboratory (Table 3). Nonetheless, the ANOVA results (Tables 31 through 34) indicate that the interlaboratory variance is significantly less than that attributed to analytical error displayed within laboratories for three of the matrices (TPH-2 soil, SRS103-100 soil, and spiked clay soil), but significantly greater than that attributed to analytical error within laboratories for the TPH-1 soil.

One-way ANOVA was also performed for the laboratories using vessels of 3.5 mL or less (Tables 35 through 38). In all four cases, we find that the interlaboratory variance is greater than that attributed to analytical error displayed within laboratories.

TABLE 27. ONE-WAY ANOVA FOR THE TPH-1 SOIL SAMPLES EXTRACTED BY ALL LABORATORIES

Laboratory code	Concentration (mg/kg) X_1	X_2	X_3	Sum
01	618	808	1,000	2,430
02	449	453	487	1,390
03	67	10	77	154
04	352	391	420	1,160
05	943	859	854	2,660
06	250	478	44	772
08	737	799	933	2,470
10	693	1,070	789	2,550
11	972	1,210	1,100	3,280
12	691	701	656	2,050
13	673	604	620	1,900
14	639	605	580	1,820
15	883	963	885	2,730
17	575	712	832	2,120
			Grand sum	27,480

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[a]
Among laboratories	13	3,114,300	239,560	19.4	2.09
Within laboratories	28	345,970	12,360		
Total	41	3,460,270			

[a] F_{crit} is $F_{0.05,\ 13,\ 28}$

TABLE 28. ONE-WAY ANOVA FOR THE TPH-2 SOIL SAMPLES EXTRACTED BY ALL LABORATORIES

Laboratory code	Concentration (mg/kg) X_1	X_2	X_3	Sum
01	1,840	2,160	1,880	5,880
02	1,940	1,990	1,730	5,660
03	257	410	356	1,020
04	1,350	1,160	1,520	4,030
05	1,490	2,000	2,230	5,720
06	1,850	1,730	1,380	4,960
08	1,480	1,100	1,500	4,080
10	1,880	2,240	1,980	6,100
11	2,140	2,200	2,050	6,390
12	2,320	2,440	2,110	6,870
13	1,900	2,010	1,940	5,850
14	2,130	1,900	1,340	5,370
15	1,850	1,900	1,990	5,740
17	1,780	1,920	1,980	5,680
			Grand sum	73,350

Source of variation	Degrees of freedom	Sum of squares	Mean square (variance)	F	F_{crit}
Before outlier removal					
Among laboratories	13	9,013,060	693,310	16.4	2.09[a]
Within laboratories	28	1,187,300	42,400		
Total	41	10,200,360	248,790		
After outlier removal					
Among laboratories	12	2,630,900	219,240	4.6	2.15[b]
Within laboratories	26	1,175,300	45,200		
Total	38	3,806,200			

[a] F_{crit} is $F_{0.05, 13, 28}$

[b] F_{crit} is $F_{0.05, 12, 26}$

TABLE 29. ONE-WAY ANOVA FOR THE SRS103-100 SOIL SAMPLES EXTRACTED BY ALL LABORATORIES

Laboratory code	Concentration (mg/kg) X_1	X_2	X_3	Sum
01	32,600	35,600	33,700	101,900
02	28,900	29,600	29,500	88,000
03	4,130	11,100	—	15,230
04	23,200	20,800	25,000	69,000
05	25,200	29,500	10,800	69,500
06	46,400	45,100	54,600	146,100
08	25,500	24,400	24,400	74,300
10	19,500	14,800	17,500	51,800
11	28,900	27,800	28,300	85,000
12	33,200	32,700	—	65,900
13	30,900	30,900	31,500	93,300
14	26,500	29,200	11,400	67,100
15	31,100	32,200	30,800	94,100
17	21,600	24,300	22,600	68,500
			Grand sum	1,085,730

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[a]
Among laboratories	13	3,052,860,000	234,835,400	13.6	2.09
Within laboratories	28	484,740,000	17,312,140		
Total	41	3,537,600,000			

[a] F_{crit} is $F_{0.05,\ 13,\ 28}$

TABLE 30. ONE-WAY ANOVA FOR THE SPIKED CLAY SOIL SAMPLES EXTRACTED BY ALL LABORATORIES

Laboratory code	Concentration (mg/kg) X_1	X_2	X_3	Sum
01	8,760	10,400	8,680	27,840
02	7,850	8,740	9,740	26,330
03	580	368	963	1,910
04	7,000	7,590	7,270	21,860
05	8,160	8,790	9,290	26,240
06	6,240	6,810	4,100	17,150
08	7,110	6,130	5,780	19,020
10	6,470	4,310	3,200	13,980
11	9,690	8,380	9,400	27,470
12	8,660	8,810	9,270	26,470
13	8,420	8,210	8,830	25,460
14	5,000	7,910	8,970	21,880
15	8,560	7,660	9,020	25,240
17	7,990	7,940	8,590	24,520
			Grand sum	305,640

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[a]
Before outlier removal					
Among laboratories	13	217,320,000	12,783,500	13.6	2.09[a]
Within laboratories	28	26,250,000	937,500		
Total	41	243,570,000			
After outlier removal					
Among laboratories	12	74,870,000	6,239,200	6.2	2.15[a]
Within laboratories	26	26,070,000	1,002,700		
Total	38	100,940,000			

[a] F_{crit} is $F_{0.05,\ 13,\ 28}$
[b] F_{crit} is $F_{0.05,\ 12,\ 26}$

TABLE 31. ONE-WAY ANOVA FOR THE TPH-1 SOIL SAMPLES EXTRACTED BY LABORATORIES 05, 13, 14, 15, AND 17

Laboratory code	Concentration (mg/kg)			Sum
	X_1	X_2	X_3	
05	943	859	854	2,660
13	673	604	620	1,900
14	639	605	580	1,820
15	883	963	885	2,730
17	575	712	832	2,120
			Grand sum	11,230

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[a]
Among laboratories	4	239,780	59,950	12.9	3.48
Within laboratories	10	46,600	4,660		
Total	14	286,380			

[a] F_{crit} is $F_{0.05, 4, 10}$

TABLE 32. ONE-WAY ANOVA FOR THE TPH-2 SOIL SAMPLES EXTRACTED BY LABORATORIES 05, 13, 14, 15, AND 17

Laboratory code	Concentration (mg/kg)			Sum
	X_1	X_2	X_3	
05	1,490	2,000	2,230	5,720
13	1,900	2,010	1,940	5,850
14	2,130	1,900	1,340	5,370
15	1,850	1,900	1,990	5,740
17	1,780	1,920	1,980	5,680
			Grand sum	28,360

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[a]
Among laboratories	4	43,290	10,820	0.16	3.48
Within laboratories	10	654,400	65,440		
Total	14	697,690			

[a] F_{crit} is $F_{0.05, 4, 10}$

TABLE 33. ONE-WAY ANOVA FOR THE SRS103-100 SOIL SAMPLES EXTRACTED BY LABORATORIES 05, 13, 14, 15, AND 17

Laboratory code	Concentration (mg/kg)			Sum
	X_1	X_2	X_3	
05	25,200	29,500	10,800	65,500
13	30,900	30,900	31,500	93,300
14	26,500	29,200	11,400	67,100
15	31,100	32,200	30,800	94,100
17	21,600	24,300	22,600	68,500
			Grand sum	388,500

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[a]
Among laboratories	4	286,050,000	71,512,500	1.9	3.48
Within laboratories	10	380,950,000	38,095,000		
Total	14	667,000,000			

[a] F_{crit} is $F_{0.05, 4, 10}$

TABLE 34. ONE-WAY ANOVA FOR THE SPIKED CLAY SOIL SAMPLES EXTRACTED BY LABORATORIES 05, 13, 14, 15, AND 17

Laboratory code	Concentration (mg/kg)			Sum
	X_1	X_2	X_3	
05	8,160	8,790	9,290	26,240
13	8,420	8,210	8,830	25,460
14	5,000	7,910	8,970	21,880
15	8,560	7,660	9,020	25,240
17	7,990	7,940	8,590	24,520
			Grand sum	123,340

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit} [a]
Among laboratories	4	3,740,160	940,720	0.9	3.48
Within laboratories	10	10,509,700	1,050,970		
Total	14	14,249,860			

[a] F_{crit} is $F_{0.05, 4, 10}$

TABLE 35. ONE-WAY ANOVA FOR THE TPH-1 SOIL SAMPLES EXTRACTED BY LABORATORIES 01, 04, 06, 08, 13, 14, AND 17

Laboratory code	Concentration (mg/kg)			Sum
	X_1	X_2	X_3	
01	618	808	1,000	2,430
04	352	391	420	1,160
06	250	478	44	772
08	737	799	933	2,470
13	673	604	620	1,900
14	639	605	580	1,820
17	575	712	832	2,120
			Grand sum	12,670

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[a]
Among laboratories	6	804,350	134,060	8.3	2.85
Within laboratories	14	227,060	16,220		
Total	20	1,031,410			

[a] F_{crit} is $F_{0.05, 6, 14}$

TABLE 36. ONE-WAY ANOVA FOR THE TPH-2 SOIL SAMPLES EXTRACTED BY LABORATORIES 01, 04, 06, 08, 13, 14, AND 17

Laboratory code	Concentration (mg/kg)			Sum
	X_1	X_2	X_3	
01	1,840	2,160	1,880	5,880
04	1,350	1,160	1,520	4,030
06	1,850	1,730	1,380	4,960
08	1,480	1,100	1,500	4,080
13	1,900	2,010	1,940	5,850
14	2,130	1,900	1,340	5,370
17	1,780	1,920	1,980	5,680
			Grand sum	35,850

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[a]
Among laboratories	6	1,260,630	210,100	3.6	2.85
Within laboratories	14	704,000	58,670		
Total	20	1,964,630			

[a] F_{crit} is $F_{0.05, 6, 14}$

TABLE 37. ONE-WAY ANOVA FOR THE SRS103-100 SOIL SAMPLES EXTRACTED BY LABORATORIES 01, 04, 06, 08, 13, AND 14[a]

Laboratory code	Concentration (mg/kg)			Sum
	X_1	X_2	X_3	
01	32,600	35,600	33,700	101,900
04	23,200	20,800	25,000	69,000
06	46,400	45,100	54,600	146,100
08	25,500	24,400	24,400	74,300
13	30,900	30,900	31,500	93,300
14	26,500	29,200	11,400	67,100
			Grand sum	552,200

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[b]
Among laboratories	5	1,496,270,000	299,254,000	14.3	3.11
Within laboratories	12	251,640,000	20,970,000		
Total	17	1,747,910,000			

[a] Laboratory 17 was not included because it used a 10-mL vessel for the extractions.

[b] F_{crit} is $F_{0.05,\ 5,\ 12}$

TABLE 38. ONE-WAY ANOVA FOR THE SPIKED CLAY SOIL SAMPLES EXTRACTED BY LABORATORIES 01, 04, 06, 08, 13, 14, AND 17

Laboratory code	Concentration (mg/kg)			Sum
	X_1	X_2	X_3	
01	8,760	10,400	8,680	27,840
04	7,000	7,590	7,270	21,860
06	6,240	6,810	4,100	17,150
08	7,110	6,130	5,780	19,020
13	8,420	8,210	8,830	25,460
14	5,000	7,910	8,970	21,880
17	7,990	7,940	8,590	24,520
			Grand sum	157,730

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	F_{crit}[a]
Among laboratories	6	27,630,000	4,605,000	4.03	2.85
Within laboratories	14	16,000,000	1,142,900		
Total	20	43,630,000			

[a] F_{crit} is $F_{0.05, 6, 14}$

Method Performance for the Clay Soil Samples Spiked with Corn Oil and Reference Oil

Table 39 shows the concentrations of oil/grease and TPHs determined in the extracts derived from spiked clay soil samples by SFE with carbon dioxide. Oil/grease is defined as the material that has been extracted from the soil sample with supercritical carbon dioxide and collected in PCE, but has not been subjected to silica gel cleanup. The spiking level was 1,000 mg/kg for each the corn oil and the reference oil. The oil/grease data indicate that six of the 14 laboratories achieved recoveries ranging from 69 to 84 percent, five laboratories had recoveries ranging from 34 to 42 percent, two had recoveries of 16 and 18 percent, and one laboratory did not submit an extract. These recoveries may be biased low because the quantification of corn oil was done against the reference oil standard. The TPH recoveries in these samples, after silica gel cleanup of the extract, ranged from 65 to 86 percent for seven laboratories, from 28 to 54 percent for four laboratories, 10 percent for two laboratories, with one laboratory not submitting an extract.

These data are difficult to interpret. We would have expected (based on the results reported earlier) much better and more consistent recoveries. It is possible that low recoveries of the reference oil are partly due to the volatilization of isooctane or chlorobenzene during extraction. We were not able to correlate these results with the flow rates of carbon dioxide in Table 9 for sample 8. For example, the laboratories using HP systems (laboratories 10, 11, and 12), with a carbon dioxide flow rate of 2 mL/min, recovered 53.6, 83, and 9.5 percent, respectively.

The data in Table 40 were obtained on the same extracts, but the IR analyses were performed with a BSci-IR spectrometer. The recoveries were slightly lower than those given in Table 39, but they followed the same trend.

Method Performance for the Wet Clay Soil Samples

Tables 41 and 42 show the method performance data for the wet clay soil samples spiked with motor oil at 10,000 mg/kg. These samples were mixed with equal portions of anhydrous sodium sulfate immediately prior to extraction. The recoveries were above 30 percent for only two laboratories (30.7 and 37.7 percent), and eight laboratories had recoveries below 8 percent.

These results indicated that additional experimental work was needed to identify a better drying method. Experiments were, therefore, performed at MRI-CO using anhydrous magnesium sulfate and Hydromatrix (diatomaceous earth) as drying agents with clay soil samples spiked with motor oil at 10,000 mg/kg. The water content of the samples was varied from 10.6 percent to 40 percent (Table 43); the extractions were performed at 340 atm/80°C/30 min (dynamic). We also extracted spiked clay soil samples containing water at 10.6, 20, 30, and 40 percent to which no drying agent was added, but the extractions were performed at 450 atm and 150°C for 30 min (dynamic). The recovery data in Table 43 indicate that at 10.6 percent water, the recoveries obtained with the two drying agents (e.g., 96 percent for anhydrous magnesium sulfate and 99 percent for Hydromatrix) were identical with those achieved at higher pressure and temperature but with no drying agent (e.g., 97 percent). As the water content increased, the recoveries decreased, and they became much lower for the experiments performed without a drying agent. Since we noticed that the clay particles tended to clump when we mixed them with anhydrous magnesium sulfate, we also used anhydrous magnesium sulfate without mixing it with the clay soil sample, but adding it as a plug in the extraction vessel such that the carbon dioxide would flow through the sample first and then through the bed of anhydrous magnesium sulfate. The recoveries achieved in those experiments were 75 percent at 20 percent

TABLE 39. RECOVERIES (PERCENT) OF OIL/GREASE AND TPHs DETERMINED IN EXTRACTS OF CLAY SOIL SPIKED WITH CORN OIL AND REFERENCE OIL (PE-FTIR SPECTROMETER)

Laboratory code	Percent recovery	
	Oil/grease	TPHs
01	71.5	71.8
02	36.6	28.5
03	42.4	76.5
04	34.1	31.0
05	b	b
06	35.3	32.2
08	16.0	10.1
10	39.5	53.6
11	78.0	83.0
12	18.1	9.5
13	73.5	78.3
14	84.0	85.8
15	70.5	65.2
17	69.0	68.4

[a] The spiking level was 1,000 mg/kg for reference corn oil and 1,000 mg/kg for reference oil. Single determinations.

[b] This laboratory did not submit an extract for this sample.

TABLE 40. RECOVERIES (PERCENT) OF OIL/GREASE AND TPHs DETERMINED IN EXTRACTS OF CLAY SOIL SPIKED WITH CORN OIL AND REFERENCE OIL (BSci-IR SPECTROMETER)[a]

Laboratory code	Percent recovery	
	Oil/grease	TPHs
01	55.0	56.4
02	30.6	25.7
03	38.4	71.3
04	25.8	23.0
05	b	b
06	29.0	26.8
08	12.5	9.1
10	30.3	52.6
11	62.0	69.0
12	14.3	10.9
13	53.5	65.8
14	68.0	70.7
15	53.5	54.3
17	55.5	56.4

[a] The spiking level was 1,000 mg/kg for reference corn oil and 1,000 mg/kg for reference oil. Single determinations.

[b] This laboratory did not submit an extract for this sample.

TABLE 41. CONCENTRATIONS (mg/kg) AND PERCENT RECOVERIES OF TPHs DETERMINED IN EXTRACTS OF WET CLAY SOIL SPIKED WITH MOTOR OIL (PE-FTIR SPECTROMETER)[a]

Laboratory code	Concentration (mg/kg)	Percent recovery
01	3,770	37.7
02	1,920	19.2
03	135	1.4
04	2,210	22.1
05	489	4.9
06	3,070	30.7
08	76.0	0.8
10	69.0	0.7
11	1,780	17.8
12	533	5.3
13	289	2.9
14	608	6.1
15	2,330	23.3
17	775	7.8

[a] The clay soil was spiked with motor oil at 10,000 mg/kg. The water content was 30 percent. Single determinations.

TABLE 42. CONCENTRATIONS (mg/kg) AND PERCENT RECOVERIES OF TPHs DETERMINED IN EXTRACTS OF WET CLAY SOIL SPIKED WITH MOTOR OIL (BSci-IR SPECTROMETER)[a]

Laboratory code	Concentration (mg/kg)	Percent recovery
01	3,730	37.3
02	1,850	18.5
03	125	1.3
04	2,150	21.5
05	489	4.9
06	2,930	29.3
08	86.0	0.9
10	71.8	0.7
11	1,740	17.4
12	561	5.6
13	316	3.2
14	612	6.1
15	2,320	23.2
17	754	7.5

[a] The clay soil was spiked with motor oil at 10,000 mg/kg. The water content was 30 percent. Single determinations.

water, but they dropped to approximately 25 to 27 percent for samples with 30 and 40 percent water (Table 43). It is possible that the low recoveries were due to restrictor plugging and subsequently to reduced flow rate of carbon dioxide.

When spiked clay soil containing 40 percent water was mixed with anhydrous magnesium sulfate (equal weights) and allowed to equilibrate overnight, or for 5 days, at room temperature, we obtained much higher recoveries of TPHs when extracting at 340 atm/80°C for 30 min (dynamic). The average recovery ± one standard deviation of eight determinations (two sets of four samples extracted in parallel) was 84.7 ± 3.4 percent for overnight equilibration (14 hours) and 76.9 ± 6.1 percent for 5-day equilibration for clay soil spiked with motor oil (Table 44), and 74.3 percent for overnight equilibration and 77.1 ± 4.6 percent for 5-day equilibration for clay soil spiked with diesel oil (Table 44). We used in both experiments a plug of anhydrous magnesium sulfate (1.5 g) and crushed the clumps that were formed upon adding magnesium sulfate. A disposable glass pipet was used for this purpose, and the crushing was done directly in the extraction vessel (to minimize losses of the more volatile petroleum hydrocarbons).

Correlation between the PE-FTIR Data and the BSci-IR Data

The development work for this method has been performed on an FTIR instrument, while the method specified the use of a filter or fixed-wavelength instrument. Questions were raised regarding how to compensate for the multiplex advantage of the FTIR and about the capability of the FTIR for spectral subtraction, which cannot be done with a filter instrument. To address these concerns, we analyzed all extracts for the interlaboratory study on two IR systems. The measurement was done first on the PE-FTIR, then the IR cuvette was placed in the BSci-IR system and the measurement was taken. The BSci-IR measurements and the PE-FTIR measurements (as TPH concentrations in mg/kg for each matrix) were then plotted on the y-axis and the x-axis, respectively. Table 45 shows the slope, intercepts, and the correlation coefficients of the FTIR and the IR data by matrix. Excellent correlation was demonstrated, as shown by the values for the correlation coefficients. The slopes indicate that the measurements obtained with the BSci-IR were always lower than those obtained with the PE-FTIR system. We cannot explain the differences; however, because these differences were 17 percent or less (the slopes of the linear regression equations ranged from 0.8267 to 0.9900 for four matrixes), we concluded that the two sets of data were comparable.

QUALITY ASSURANCE/QUALITY CONTROL

The quality control data generated in this study are presented in Tables 46 through 56. They include all calibration data generated during the analysis of extracts (by instrument, by material used in calibration, and by date) (Tables 46 through 52), the results from the analyses of the unspiked clay soil samples (Table 53), the results of the analyses of the PCE blanks (Table 54), the results from the analysis of all system blanks submitted by the participating laboratories (Table 55), and the results of the sample storage study (Table 56).

TABLE 43. PERCENT RECOVERIES OF TPHs DETERMINED IN EXTRACTS OF WET CLAY SOIL SPIKED WITH MOTOR OIL (PE-FTIR SPECTROMETER)[a]

Percent water	Percent recovery			
	Addition of anhydrous $MgSO_4$[b]		Addition of Hydromatrix[b]	No drying agent[c]
	Plug only	Mixed with sample		
10.6	--	95.9 ± 1.2	98.8	96.8
20	74.9	86.4 ± 9.2	72.2	74.8 ± 12.2
30	24.8	55.8	53.1	52.0
40	26.9	45.3	47.1	29.2

[a] The value given is the average recovery ± one standard deviation (for triplicate determinations) or the average recovery of duplicate determinations.
[b] The extraction was performed at 340 atm/80°C/30 min (dynamic).
[c] The extraction was performed at 450 atm/150°C/30 min (dynamic).

TABLE 44. PERCENT RECOVERIES OF TPHs DETERMINED IN EXTRACTS OF WET CLAY SOIL (40 PERCENT WATER) SPIKED WITH MOTOR OIL OR DIESEL OIL (PE-FTIR SPECTROMETER)[a]

Storage Condition	Motor oil	Diesel oil
14 hours at 22°C	84.7 ± 3.4[b]	--
120 hours at 22°C	76.9 ± 6.1[c]	--
14 hours at 4°C	--	74.3[d]
120 hours at 4°C	--	77.1 ± 4.6[e]

[a] The extractions were performed at 340 atm/80°C/30 min (dynamic). Each spiked sample (3 g) was mixed with 3 g of anhydrous magnesium sulfate and stored as indicated. For extraction, a plug of 1.5 g of anhydrous sodium sulfate was put into each extraction vessel at the outlet of the vessel.
[b] The number of determinations was eight.
[c] The number of determinations was seven.
[d] Duplicate determination.
[e] The number of determinations was three.

TABLE 45. CORRELATION BETWEEN THE PE-FTIR DATA AND THE BSci-IR DATA[a]

Matrix	Sample identification	Slope	Intercept	Correlation coefficient	Number of data points
TPH-1 soil	1	0.9900	-29.5	0.9929	42
TPH-2 soil	2	0.8711	50.3	0.9953	42
SRS103-100	3	0.8880	945	0.9975	40
Spiked clay soil	4,6,7	0.9767	534	0.9831	42
Spiked clay soil (oil/grease analysis)	8	0.7689	21.8	0.9927	13
Spiked clay soil (TPH analysis)	8	0.8271	13.9	0.9891	13
Wet clay soil	9	0.9727	12.4	0.9997	14

[a] The BSci-IR data were plotted on the y-axis and the PE-FTIR data were plotted on the x-axis.

TABLE 46. CALIBRATION DATA FOR THE PE-FTIR SPECTROMETER USING REFERENCE OIL IN PCE AND THE 10-mm PATH-LENGTH IR CELL[a]

Date	Concentration (μg/mL)	Absorbance	Slope	Intercept	Correlation coefficient
04/03/92	10	0.0560	0.0018	-0.0016	0.9996
	25	0.0406			
	50	0.0856			
	100	0.1797			
	250	0.4525			
	500	0.8672			
04/06/92	10	0.0225	0.0017	0.0314	0.9987
	25	0.0725			
	50	0.1220			
	100	0.2222			
	250	0.4736			
	500	0.8768			
04/07/92	10	0.0361	0.0018	0.0288	0.9992
	25	0.0748			
	50	0.1085			
	100	0.2109			
	250	0.4921			
	500	0.8992			
04/10/92	10	-0.0058	0.0017	0.0121	0.9982
	25	0.0785			
	50	0.1056			
	100	0.1899			
	250	0.4491			
	500	0.8722			
04/16/92	10	0.0242	0.0017	0.0247	0.9992
	25	0.0718			
	50	0.1082			
	100	0.2102			
	250	0.4749			
	500	0.8842			
04/17/92	10	0.0239	0.0017	0.0242	0.9992
	25	0.0715			
	50	0.1070			
	100	0.2092			
	250	0.4746			
	500	0.8828			

(continued)

[a] Silica gel was added to the calibration standards.

TABLE 46. (concluded)[a]

Date	Concentration (μg/mL)	Absorbance	Slope	Intercept	Correlation coefficient
04/21/92	10	0.0240	0.0017	0.0247	0.9992
	25	0.0713			
	50	0.1089			
	100	0.2115			
	250	0.4759			
	500	0.8869			
04/23/92	10	0.0240	0.0017	0.0242	0.9993
	25	0.0706			
	50	0.1070			
	100	0.2087			
	250	0.4738			
	500	0.8813			
04/24/92	10	0.0236	0.0017	0.0245	0.9993
	25	0.0715			
	50	0.1101			
	100	0.2097			
	250	0.4725			
	500	0.8848			
04/28/92	10	0.0237	0.0017	0.0234	0.9993
	25	0.0704			
	50	0.1074			
	100	0.2089			
	250	0.4731			
	500	0.8850			
05/01/92	10	0.0230	0.0017	0.0226	0.9994
	25	0.0704			
	50	0.1077			
	100	0.2086			
	250	0.4743			
	500	0.8903			
05/05/92	10	0.0231	0.0017	0.0234	0.9993
	25	0.0701			
	50	0.1066			
	100	0.2089			
	250	0.4722			
	500	0.8814			

[a] Silica gel was added to the calibration standards.

TABLE 47. CALIBRATION DATA FOR THE BSci-IR SPECTROMETER USING REFERENCE OIL IN PCE AND THE 10-mm PATH-LENGTH IR CELL[a]

Date	Concentration (μg/mL)	Absorbance	Slope	Intercept	Correlation coefficient
04/03/92	10	0.006	0.0016	-0.0074	1.000
	25	0.033			
	50	0.073			
	100	0.156			
	250	0.400			
	500	0.799			
04/06/92	10	0.020	0.0016	0.0260	0.9992
	25	0.072			
	50	0.109			
	100	0.198			
	250	0.422			
	500	0.813			
04/07/92	10	0.031	0.0016	0.0197	0.9998
	25	0.067			
	50	0.093			
	100	0.183			
	250	0.433			
	500	0.826			
04/10/92	10	-0.003	0.0016	0.0055	0.9985
	25	0.069			
	50	0.092			
	100	0.165			
	250	0.393			
	500	0.805			
04/16/92	10	0.015	0.0016	0.0112	0.9997
	25	0.058			
	50	0.089			
	100	0.178			
	250	0.412			
	500	0.806			
04/17/92	10	0.024	0.0016	0.0162	0.9998
	25	0.061			
	50	0.093			
	100	0.182			
	250	0.416			
	500	0.811			

(continued)

[a] Silica gel was added to the calibration standards.

TABLE 47. (concluded)[a]

Date	Concentration (μg/mL)	Absorbance	Slope	Intercept	Correlation coefficient
04/21/92	10	0.019	0.0016	0.0143	0.9998
	25	0.060			
	50	0.093			
	100	0.182			
	250	0.417			
	500	0.813			
04/23/92	10	0.019	0.0016	0.0147	0.9997
	25	0.061			
	50	0.093			
	100	0.181			
	250	0.415			
	500	0.809			
04/24/92	10	0.022	0.0016	0.0184	0.9997
	25	0.064			
	50	0.098			
	100	0.185			
	250	0.419			
	500	0.813			
04/28/92	10	0.012	0.0016	0.0075	0.9997
	25	0.054			
	50	0.085			
	100	0.172			
	250	0.404			
	500	0.796			
05/01/92	10	0.022	0.0016	0.0179	0.9997
	25	0.063			
	50	0.096			
	100	0.187			
	250	0.420			
	500	0.815			
05/05/92	10	0.022	0.0016	0.0190	0.9996
	25	0.068			
	50	0.095			
	100	0.185			
	250	0.417			
	500	0.810			

[a] Silica gel was added to the calibration standards.

TABLE 48. CALIBRATION DATA FOR THE PE-FTIR SPECTROMETER USING MOTOR OIL IN PCE AND THE 10-mm PATH-LENGTH IR CELL[a]

Date	Concentration (μg/mL)	Absorbance	Slope	Intercept	Correlation coefficient
04/14/92	10	0.0306	0.0022	0.0263	0.9984
	25	0.0701			
	50	0.1323			
	100	0.2576			
	250	0.6152			
	500	1.0992			
04/17/92	10	0.0237	0.0022	0.0175	0.9987
	25	0.0629			
	50	0.1236			
	100	0.2499			
	250	0.6050			
	500	1.0987			
04/22/92	10	0.0252	0.0021	0.0281	0.9963
	25	0.0640			
	50	0.1257			
	100	0.2515			
	250	0.6159			
	500	1.0310			
04/30/92	10	0.0251	0.0022	0.0189	0.9987
	25	0.0640			
	50	0.1258			
	100	0.2514			
	250	0.6072			
	500	1.1016			
05/05/92	10	0.0255	0.0022	0.0182	0.9989
	25	0.0643			
	50	0.1249			
	100	0.2508			
	250	0.6020			
	500	1.1023			

[a] Silica gel was added to the calibration standards.

TABLE 49. CALIBRATION DATA FOR THE BSci-IR SPECTROMETER USING MOTOR OIL IN PCE AND THE 10-mm PATH-LENGTH IR CELL[a]

Date	Concentration (μg/mL)	Absorbance	Slope	Intercept	Correlation coefficient
04/14/92	10	0.030	0.0020	0.0115	0.9999
	25	0.057			
	50	0.109			
	100	0.215			
	250	0.516			
	500	1.002			
04/17/92	10	0.020	0.0020	0.0031	1.000
	25	0.052			
	50	0.103			
	100	0.206			
	250	0.506			
	500	1.000			
04/22/92	10	0.020	0.0020	0.0014	1.000
	25	0.050			
	50	0.101			
	100	0.205			
	250	0.502			
	500	1.001			
04/30/92	10	0.021	0.0020	0.0024	1.000
	25	0.051			
	50	0.101			
	100	0.206			
	250	0.505			
	500	1.001			
05/05/92	10	0.021	0.0020	0.0037	1.000
	25	0.053			
	50	0.103			
	100	0.207			
	250	0.506			
	500	1.002			

[a] Silica gel was added to the calibration standards.

TABLE 50. CALIBRATION DATA FOR THE PE-FTIR SPECTROMETER USING REFERENCE OIL IN PCE AND THE 10-mm PATH-LENGTH IR CELL (NO SILICA GEL CLEANUP)[a]

Date	Concentration (μg/mL)	Absorbance	Slope	Intercept	Correlation coefficient
04/16/92	10	0.0305	0.0017	0.0192	0.9989
	25	0.0559			
	50	0.0977			
	100	0.1959			
	250	0.4784			
	500	0.8652			
04/23/92	10	0.0225	0.0017	0.0146	0.9992
	25	0.0497			
	50	0.0960			
	100	0.1936			
	250	0.4597			
	500	0.8494			
04/28/92	10	0.0202	0.0017	0.0121	0.9992
	25	0.0479			
	50	0.0933			
	100	0.1897			
	250	0.4569			
	500	0.8461			
05/01/92	10	0.0214	0.0017	0.0132	0.9992
	25	0.0484			
	50	0.0941			
	100	0.1920			
	250	0.4576			
	500	0.8470			
05/05/92	10	0.0208	0.0017	0.0101	0.9993
	25	0.0476			
	50	0.0944			
	100	b			
	250	0.4558			
	500	0.8468			

[a] No silica gel was added to the calibration standards.
[b] Calibration was not performed with the 100-μg/mL standard.

TABLE 51. CALIBRATION DATA FOR THE BSci-IR SPECTROMETER USING REFERENCE OIL IN PCE AND THE 10-mm PATH-LENGTH IR CELL (NO SILICA GEL CLEANUP)[a]

Date	Concentration (μg/mL)	Absorbance	Slope	Intercept	Correlation coefficient
04/16/92	10	0.027	0.0016	0.0081	0.9998
	25	0.046			
	50	0.084			
	100	0.171			
	250	0.429			
	500	0.820			
04/23/92	10	0.019	0.0016	0.0046	0.9999
	25	0.042			
	50	0.083			
	100	0.169			
	250	0.411			
	500	0.804			
04/28/92	10	0.018	0.0016	0.0049	0.9999
	25	0.043			
	50	0.083			
	100	0.169			
	250	0.410			
	500	0.801			
05/01/92	10	0.019	0.0016	0.0051	0.9999
	25	0.043			
	50	0.083			
	100	0.171			
	250	0.412			
	500	0.806			
05/05/92	10	0.019	0.0016	0.0032	0.9999
	25	0.041			
	50	0.083			
	100	b			
	250	0.411			
	500	0.804			

[a] No silica gel was added to the calibration standards.
[b] Calibration was not performed with the 100-μg/mL standard.

TABLE 52. CONCENTRATIONS OF DAILY STANDARDS ANALYZED TO VERIFY SYSTEM REPRODUCIBILITY

	Concentration (μg/mL)[a]	
Date of analysis	PE-FTIR	BSci-IR
04/06/92	110	103
04/06/92	104	108
04/06/92	104	108
04/09/92	100	103
04/09/92	100	100
04/10/92	105	106
04/10/92	107	108
04/10/92	112	115
04/10/92	107	111
04/14/92	112	108
04/14/92	112	112
04/14/92	106	106
04/14/92	101	100
04/16/92	107	105
04/16/92	100	102
04/16/92	107	105
04/16/92	105	103
04/17/92	105	103
04/21/92	106	107
04/21/92	104	107
04/23/92	105	103
04/23/92	104	103
04/23/92	106	106
04/24/92	107	103
04/28/92	106	105
04/30/92	105	104
04/30/92	105	103
05/01/92	106	102
05/01/92	105	108
05/01/92	104	109
05/05/92	107	104

[a] The true concentration of the calibration standard was 100 μg/mL.

TABLE 53. CONCENTRATIONS OF TPHs IN THE EXTRACTS FROM THE UNSPIKED CLAY SOIL SAMPLES

	Concentration (mg/kg)	
Laboratory	PE-FTIR	BSci-IR
01	207	204
02	< 10	< 10
03	492	469
04	43	60
05	306	305
06	103	88
08	a	a
10	423	422
11	204	196
12	58	54
13	325	327
14	398	400
15	328	332
17	251	250

[a] Extract not submitted for analysis.

TABLE 54. CONCENTRATIONS OF TPHs IN THE PCE BLANKS ANALYZED DURING THIS STUDY

Date of analysis	Number of blanks performed during day	Concentration (μg/mL PCE)	
		PE-FTIR	BSci-IR
04/03/92	3	< 10	< 10
04/06/92	5	< 10	< 10
04/09/92	3	< 10	< 10
04/10/92	4	< 10	< 10
04/14/92	5	< 10	< 10
04/16/92	2	< 10	< 10
04/17/92	1	< 10	< 10
04/21/92	2	< 10	< 10
04/23/92	2	< 10	< 10
04/24/92	1	< 10	< 10
04/28/92	1	< 10	< 10
04/30/92	2	< 10	< 10
05/01/92	3	< 10	< 10
05/05/92	3	< 10	< 10

TABLE 55. CONCENTRATIONS OF TPHs IN THE EXTRACTS SUBMITTED AS SYSTEM BLANKS

Laboratory	Number of blanks submitted	Concentration (mg/mL)	
		PE-FTIR	BSci-IR
01	0	a	a
02	1	< 10	< 10
03	2	< 10; < 10	< 10; < 10
04	1	< 10	< 10
05	2	< 10; < 10	< 10; < 10
06	0	a	a
08	1	< 10	< 10
10	0	a	a
11	5	229; 18; 96; < 10; 37	238; 22; 98 27; 41
12	0	a	a
13	1	81	62
14	4	22; < 10; < 10; < 10	18; < 10; < 10; 11
15	4	10; < 10; < 10; < 10	14; < 10; < 10; < 10
17	5	< 10; < 10; < 10; < 10; < 10	< 10; < 10; < 10; < 10; < 10;

[a] Extract not submitted for analysis.

TABLE 56. PERCENT RECOVERIES OF TPHs FROM THE SPIKED CLAY SOIL SAMPLES, STORED AT 4°C IN THE DARK, AS A FUNCTION OF TIME

Days of storage	PE-FTIR		BSci-IR	
22	83.1	79.1	87.2	82.2
34	85.6	76.6	87.7	81.2
40	87.4	77.9	90.7	81.7

[a] The clay soil samples were spiked with motor oil at 10,000 mg/kg. The values given are for duplicate determinations at each time.

The following conclusions can be drawn from these data:

- The PE-FTIR and the BSci-IR calibration data generated during this study agreed to within 10 percent. The slopes for the reference oil calibrations were 0.0017 for the PE-FTIR system and 0.0016 for the BSci-IR system, and the correlation coefficients for standards ranging from 10 to 500 μg/mL were 0.999 or greater (Tables 46 and 47). The slopes for the motor oil calibrations were 0.0022 for the PE-FTIR system (Table 48) and 0.0020 for the BSci-IR system (Table 49).

- Both IR spectrometer systems gave reproducible results over the period of 1 month during which IR analyses were performed. The multilevel calibration data (slopes, intercepts, and correlation coefficients) from each instrument indicate deviations less than 10 percent for the IR determinations performed over a period of 1 month. A 100-μg/mL reference oil or motor oil standard was analyzed after every 10 analyses. The largest deviation (+15 percent) was found on one day with the BSci-IR system. The other deviations averaged approximately 5 percent over a period of 1 month (Table 52).

- The extracts from the unspiked clay soil samples (sample 5) did not contain TPHs above 10 mg/kg. We analyzed this material in our laboratory on several occasions and found TPHs at levels ranging from 5 to 9 mg/kg. The sample-5 extracts submitted by the participating laboratories, however, contained TPHs at concentrations as high as 492 mg/kg (Table 53). This implies that the levels reported for sample 5 are due to cross-contamination from the previous extraction, since we instructed the laboratories to analyze the clay soil blank immediately after the spiked clay soil (concentration 10,000 mg/kg). Thus, contamination of the SFE system, especially when dealing with high-concentration samples, is likely, and the analyst must take the necessary precautions to minimize it (e.g., clean extraction vessel and frits, replace restrictor, perform system blanks).

- The Aldrich PCE (spectrophotometric grade) was acceptable for this study. The solvent blanks analyzed during the study indicated TPH levels of less than 10 μg/mL (Table 54).

- The system blanks generated with the Aldrich PCE and the SFE-grade carbon dioxide did not show contamination, with the exception of laboratories 11 and 13 (Table 55). Since both laboratories used the Scott SFE-grade carbon dioxide, and since the PCE was provided by MRI-CO, it appears that the source of contamination was in the SFE system.

- The percent recoveries from the spiked clay soil samples, that had been stored at 4°C in the dark and extracted after 22, 34, and 44 days, were independent of the storage time.

REFERENCES

1. Lopez-Avila, V., N. S. Dodhiwala, J. Benedicto, and R. Young, "SFE/IR Method for the Determination of Petroleum Hydrocarbons in Soils and Sediments," EPA 600/X-92/046, April 1992 (W.F. Beckert, Project Officer, EMSL-LV).

2. Lopez-Avila, V., J. Benedicto, N. S. Dodhiwala, and W. F. Beckert, "Development of an Off-Line SFE/IR Method for Petroleum Hydrocarbons in Soils," J. Chromatogr. Sci. 30, 335-343, 1992.

3. Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis, J. Assoc. Off. Anal. Chem. 72, 694-704, 1989.

4. "Lotus Spreadsheet Program for the Calculation of Performance Parameters from Collaborative Study Data Including Outlier Analyses," revision 3/5/91, received from John G. Phillips, Chairman, AOAC Statistics Committee.

5. EPA Method 418.1, "Total Recoverable Petroleum Hydrocarbons," in "Methods for Chemical Analyses of Water and Wastewater," EPA 600/14-79/020, revised 1983.

APPENDIX A

PROPOSED DRAFT PROTOCOL FOR SUPERCRITICAL FLUID EXTRACTION OF PETROLEUM HYDROCARBONS (METHOD 3560)

METHOD 3560

SUPERCRITICAL FLUID EXTRACTION OF TOTAL RECOVERABLE PETROLEUM HYDROCARBONS

1.0 SCOPE AND APPLICATION

1.1 Method 3560 describes the extraction with supercritical fluids of total recoverable petroleum hydrocarbons (TPHs) from soils, sediments, fly ash, and other solid materials that are amenable to extraction with conventional solvents. The method is suitable for use with any supercritical fluid extraction (SFE) system that allows extraction conditions (e.g., pressure, temperature, flow rate) to be adjusted to achieve separation of the TPHs from the matrices of concern.

1.2 Method 3560 is not suitable for the extraction of low-boiling TPHs such as gasoline.

2.0 SUMMARY OF METHOD

2.1 A known amount of sample is transferred to the extraction vessel. The sample is then extracted in the dynamic mode for up to 30 min with supercritical carbon dioxide at 340 atm and 80°C and a gas flow rate of 500 to 1,000 mL/min. After depressurization of the carbon dioxide, the extracted TPHs are collected in 3 mL of tetrachloroethylene or other appropriate solvent (see Section 5.3), or on a sorbent material, depending on the SFE system used. In the latter case, the analytes are collected by rinsing the sorbent material with tetrachloroethylene or other suitable solvent. After collection, the TPHs are analyzed by the appropriate determinative method.

3.0 INTERFERENCES

3.1 The analyst must demonstrate through the analysis of reagent blanks (collection solvent treated as per Section 7.4) that the supercritical fluid extraction system is free of interferents. To do this, perform a simulated extraction using an empty extraction vessel and a known amount of carbon dioxide under the same conditions as those used for sample extraction, and determine the background contamination by analyzing the extract by the appropriate determinative method (e.g., Method 8015 or 8440). If glass wool and a drying agent are used with the sample, they should be included when performing a reagent blank check.

3.2 The extraction vessel(s), the frits, the restrictor(s), and the multiport valve may retain solutes whenever high-concentration samples are extracted. It is, therefore, good practice to clean the extraction system after each extraction. Replacement of the restrictor may be necessary when reagent blanks indicate carryover. At least one reagent blank should be prepared and analyzed daily when the instrument is in use. Furthermore, reagent blanks should be prepared and analyzed after each extraction of a high-concentration sample (concentration in the high ppm range). If reagent blanks continue to indicate contamination even after replacement of the extraction vessel and the restrictor, the multi-port valve must be cleaned.

4.0 APPARATUS AND MATERIALS

4.1 Supercritical fluid extractor and associated hardware.

WARNING - A safety feature to prevent overpressurization is required on the extractor. This feature should be designed to protect the laboratory personnel and the instrument from possible injuries or damage resulting from equipment failure under high pressure.

4.1.2 Extraction vessel -- Stainless-steel vessel with end fittings and 0.5- or 2-μm frits. Use the extraction vessel recommended by the manufacturer of the SFE system being used. The volume of the extraction vessel should fit the sample size. PEEK (polyether ether ketone) extraction vessels are acceptable only for use with specifically designed instruments.

4.1.2.1 Fittings used for the extraction vessel must be capable of withstanding the required extraction pressures. The maximum operating pressure for most extractors is 500 atm; however, extractors with higher pressure ratings are available. Check with the manufacturer of the particular extraction system on the maximum operating pressure and temperature for that system. Make sure that the extraction vessels are rated for such pressures and temperatures.

4.1.3 Restrictor -- 50-μm ID x 150- or 375-μm OD x 25- to 60-cm length piece of uncoated fused-silica tubing (J&W Scientific or equivalent). Other restrictors may be used including tapered restrictors, static pinhole restrictors, frit restrictors, and variable orifice restrictors (manual and computer-controlled) or crimped metal tubing. Check with the manufacturer of the SFE system on the advantages and disadvantages of the various restrictor designs.

4.1.4 Collection device -- The extracted TPHs can be collected either in vials containing solvent, or they can be trapped on a sorbent material (e.g., octadecyl-bonded silica, stainless-steel beads).

4.1.4.1 When the analytes are collected in a solvent, install the restrictor through a hole made through the cap and septum of the vial, and position the restrictor end about 0.5 inch from the bottom of the vial. A syringe needle should also be inserted through the septum of the vial (with the tip positioned just below the septum) to prevent buildup of pressure in the vial. Use the type of vials appropriate for the SFE system used.

4.1.4.2 When the analytes are trapped on a sorbent material, it is important to ensure that breakthrough of the analytes from the trap does not occur. Desorption from the trapping medium can be accomplished by increasing the temperature of the trap and using a solvent to remove the analytes. To recover the analytes, use the conditions suggested by the manufacturer of the particular system used.

4.2 Carbon dioxide cylinder balance (optional) -- Balances from White Associates, catalog no. 30, Scott Specialty Gases Model 5588D, or equivalent, can be used to monitor the fluid usage. Such a device is useful because carbon dioxide tanks used for SFE are not equipped with regulators, and it is difficult to determine when the tank needs to be replaced.

4.3 Tools required include: screwdriver (flat-blade), adjustable wrench, pliers, tubing cutter, and various small open-end wrenches for small fittings.

4.4 Other materials -- Magnesium sulfate monohydrate can be used as received. The following materials require high-temperature treatment (muffling at 400°C for 2 to 4 hours) prior to use since they may be contaminated with petroleum hydrocarbons. Alternatively, a blank can be performed to determine whether these materials are sufficiently clean.

4.4.1 Silanized glass wool.

4.4.2 Drying agents such as anhydrous magnesium sulfate or diatomaceous earth.

5.0 REAGENTS

5.1 Carbon dioxide, CO_2 -- Either supercritical fluid chromatography (SFC-grade) or SFE-grade CO_2 is acceptable for use in SFE. Aluminum cylinders are preferred over steel cylinders. The cylinders are fitted with eductor tubes, and their contents are under 1500 psi of helium head pressure.

5.2 Carbon dioxide (CO_2) for cryogenic cooling -- Certain parts of some models of extractors (i.e., the high-pressure pump head and the analyte trap) must be cooled during use. The carbon dioxide used for this purpose must be dry (< 50 ppm water content), and it must be supplied in tanks with a full-length eductor tube.

5.3 Tetrachloroethylene, C_2Cl_4 (spectrophotometric grade) -- Used for the collection of TPHs for determination by IR. Analyze a blank to ensure no interferences are present at the TPH wavelengths. Chlorofluorocarbons are not suitable for use with this method because of the risk to the ozone layer.

5.4 Other appropriate pesticide-quality solvents may be used for the collection of TPHs for determination by GC (i.e., methylene chloride). Chlorofluorocarbons are not suitable for use with this method because of the risk to the ozone layer.

5.5 Copper filings -- Copper filings added to remove elemental sulfur must have a shiny bright appearance to be effective. To remove oxides from copper surfaces, treat with dilute nitric acid, rinse with reagent water to remove all traces of acid, rinse with acetone (copper will darken if acid is still present), and dry under a stream of nitrogen.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Solid samples should be collected and stored in the same manner as any other solid samples containing semivolatile organics. See Chapter Four for guidance relating to semivolatile organics (including holding times).

7.0 PROCEDURE

7.1 Determination of sample percent dry weight -- In certain cases, sample results are desired based on dry-weight basis. When such data are desired, a portion of sample for this determination should be weighed out at the same time as the portion used for analytical determination.

WARNING: The drying oven should be contained in a hood or vented.. Significant laboratory contamination may result from a heavily contaminated hazardous waste sample.

7.1.1 Immediately after weighing the sample portion for extraction, weigh 5.00 to 10.00 g of the remaining sample into a tared crucible. Determine the percent dry weight of the sample by drying it overnight at 105°C. Allow it to cool in a desiccator before weighing. Calculate the percent dry weight as follows:

$$\% \text{ dry weight} = \frac{\text{g of dry sample} \times 100}{\text{g of sample}}$$

7.2 Safety considerations -- Read section 11.0 "Safety" before attempting this procedure.

7.3 Sample handling

7.3.1 Decant and discard any aqueous layer that has accumulated on a sediment sample. Mix the sample thoroughly, especially composited samples. Discard any foreign objects such as sticks, leaves, and rocks.

7.3.2 Weigh 3 g of sample into a precleaned aluminum dish. A drying agent (e.g., anhydrous magnesium sulfate or diatomaceous earth) may be added to samples that contain water in excess of 20% to increase porosity or to bind water. Alternatively, magnesium sulfate monohydrate is an excellent drying agent, and the amount of heat released (compared with anhydrous magnesium sulfate) is small, thereby minimizing the loss of volatile petroleum hydrocarbons. The amount of the drying agent will depend on the water content of the sample. Typically, a ratio of 1:1 works well for wet soils and sediment materials. However, a certain amount of water (up to 10%) in the sample has been shown to improve recoveries from certain matrices; therefore, if the sample is dry, water may optionally be added to bring the water content to approximately 20%.

7.3.2.1 If drying agent has been added to the sample, store the mixture of sample and drying agent for several hours (preferably overnight) at 4°C, with a minimum of headspace. This additional storage time is necessary to achieve acceptable analyte recovery.

7.3.3 Transfer the weighed sample to a clean extraction vessel. The volume of the extraction vessel should match the sample volume. Use two plugs of silanized glass wool to hold the sample in place and fill the void volume (the use of drying agent or clean sand after the second glass wool plug to fill the void volume is also recommended). Attach the end fittings, and install the extraction vessel in the oven. Always use clean frits for each extraction vessel.

7.4 Sample extraction

7.4.1 Fill the collection vessel with 3 mL of tetrachloroethylene or other appropriate collection solvent. Chlorofluorocarbons are not suitable for use with this method because of the risk to the ozone layer.

7.4.2 Set the pressure at 340 atm and the temperature at 80°C. Follow manufacturer's instructions in setting up the instrument. Extract for 30 minutes in the dynamic mode. Note the safety precautions in Section 11 on venting the instrument into a chemical fume hood.

7.4.3 After the extraction time has elapsed, the system should automatically go to the equilibrate mode. At this point, remove the collection vessel(s) containing the extract(s). Since the depressurization of the carbon dioxide at the end of the restrictor outlet results in a gas flow rate of about 500 to 1000 mL/min, part of the collection solvent will evaporate during the extraction. However, cooling caused by the rapid expansion of the carbon dioxide limits the loss of solvent, so that approximately 2 mL remains (when tetrachloroethylene is used) after a 30-min extraction. To prevent the collection solvent from freezing during the extraction, place the collection vial in a beaker with warm water (approximately 25°C). The extract is then brought to the desired volume, or concentrated further. See Method 3510 for concentration techniques by micro Kuderna-Danish or nitrogen blowdown. Concentration must be performed in a chemical fume hood to prevent contamination of the laboratory environment.

7.4.4 Record the volume of liquid carbon dioxide used for extraction. Calculate the average flow rate by dividing the volume of the carbon dioxide by the extraction time.

7.4.5 The extract is ready for analysis by Method 8015 -- Nonhalogenated Volatile Organics by Gas Chromatography, or by Method 8440 -- Total Recoverable Petroleum Hydrocarbons by Infrared Spectrophotometry.

7.5 SFE System Maintenance

7.5.1 Depressurize the system following manufacturer's instructions.

7.5.2 After extraction of an especially tarry sample, the frits may require replacement to ensure adequate extraction fluid flow through the restrictor. In addition, very fine particles contained in samples can clog the frits, necessitating replacement.

7.5.3 Clean the extraction vessel after each sample. The cleaning procedure depends on the type of sample. After removing the bulk of the extracted sample from the extraction vessel, the vessel should be scrubbed with an ionic detergent, water, and a bottle brush. After extraction of tarry materials, use solvent rinses or an ultrasonic bath to clean the extraction vessel.

7.5.4 For samples known to contain elemental sulfur, use copper filings to remove the dissolved sulfur from the fluid. The copper filings (1 to 2 g per sample) can be packed in a separate extraction vessel connected to the outlet end of the sample extraction vessel, or they can be mixed with the sample, and a plug of copper filings can be loaded in the extraction

vessel with the sample such that any sulfur extracted by the carbon dioxide can be removed before the stream of carbon dioxide containing the analytes reaches the restrictor.

7.5.5 The procedure to be followed in emptying the syringe pump depends upon the type of fluid being used. In the case of carbon dioxide, which is a gas at ambient temperature and pressure, it is only necessary to vent the gas to a fume hood by allowing it to expand across the purge valve. Follow manufacturer's instructions in emptying the syringe pump.

7.5.6 To change fluid supply cylinders, follow manufacturer's instructions.

7.5.7 Restrictor removal and installation -- Follow manufacturer's instructions. When using fused-silica restrictors, it may be necessary to replace the restrictor after each sample, especially when extracting samples contaminated with heavy oils.

8.0 QUALITY CONTROL

8.1 Reagent blanks or matrix-spiked samples must be subjected to the same analytical procedures (Section 7.4) as those used on actual samples.

8.2 Refer to Chapter One for specific Quality Control procedures and to Method 3500 for sample preparation quality control procedures.

8.3 All instrument operating conditions must be recorded.

9.0 METHOD PERFORMANCE

9.1 Refer to Method 8440 and 8015 for performance data.

9.2 Use standard reference materials to establish the performance of the method with contaminated samples.

10.0 REFERENCES

1. Lopez-Avila, V., N. S. Dodhiwala, J. Benedicto, and R. Young, "SFE/IR Method for the Determination of Petroleum Hydrocarbons in Soils and Sediments," EPA 600/X-92/046 (W.F. Beckert, Project Officer), US EPA, Environmental Monitoring Systems Laboratory, Las Vegas, April 1992.

2. Pyle, S. M., and M. M. Setty, "Supercritical Fluid Extraction of High-Sulfur Soils with Use of a Copper Scavenger," Talanta, 1991, 38 (10), 1125-1128.

11.0 SAFETY

11.1 When liquid carbon dioxide comes in contact with skin, it can cause burns because of its low temperature. Burns are especially severe when the carbon dioxide is modified with organic liquids.

11.2 The extraction fluid, which may contain a modifier, usually exhausts through an exhaust gas port on the rear of the panel of the extractor. This port must be connected to a chemical fume hood to prevent contamination of the laboratory atmosphere.

11.3 When liquid carbon dioxide is used for cryogenic cooling, typical coolant consumption is 5 L/min (as decompressed gas), which results in a carbon dioxide level of 900 ppm for a room of 4.5 m x 3.0 m x 2.5 m, assuming 10 air exchanges per hour. The NIOSH time-weighted average concentration is 9,000 ppm (American Conference of Governmental Industrial Hygienists, 1991-1992).

APPENDIX B

PROPOSED DRAFT PROTOCOL FOR DETERMINATION OF TOTAL RECOVERABLE PETROLEUM HYDROCARBONS BY INFRARED SPECTROPHOTOMETRY (METHOD 8440)

METHOD 8440

TOTAL RECOVERABLE PETROLEUM HYDROCARBONS BY INFRARED SPECTROPHOTOMETRY

1.0 SCOPE AND APPLICATION

1.1 Method 8440 (formerly Method 9073) is used for the measurement of total petroleum hydrocarbons (TPHs) extracted with supercritical carbon dioxide from sediment, soil, and sludge samples using Method 3560.

1.2 Method 8440 is not applicable to the measurement of gasoline.

1.3 Method 8440 can detect TPHs at concentrations of 10 μg/mL in extracts. This translates to 10 mg/Kg in soils when a 3-g sample is extracted by SFE (assuming 100 percent extraction efficiency) and the final extract volume is 3 mL.

1.4 All organic solvents used in this method should be recovered and recycled.

2.0 SUMMARY OF METHOD

2.1 Soil samples are extracted with supercritical carbon dioxide using Method 3560. Interferences are removed with silica gel, either by shaking the extract with loose silica gel, or by passing it through a silica gel solid-phase extraction cartridge. After infrared (IR) analysis of the extract, TPHs are quantified by direct comparison with standards.

3.0 INTERFERENCES

3.1 The parameter being measured (TPHs) is defined within the context of this method. The measurement may be subject to interferences, and the results should be interpreted accordingly.

3.2 Determination of TPHs is a gross measure of mineral oils only, and does not include the biodegradable animal greases and vegetable oils captured in oil-and-grease measurements. These non-mineral-oil contaminants may cause positive interferences with the IR analysis if they are not completely removed by the silica gel cleanup.

4.0 APPARATUS AND MATERIALS

4.1 Infrared spectrophotometer -- Scanning or fixed wavelength, for measurement around 2950 cm^{-1}.

4.2 IR cells -- 10-mm, 50-mm, and 100-mm path length, made of sodium chloride or IR-grade glass.

4.3 Optional -- A vacuum manifold consisting of glass vacuum basin, collection rack and funnel, collection vials, replaceable stainless steel delivery tips, built-in vacuum bleed valve and gauge

is recommended for use when silica gel cartridges are used. The system is connected to a vacuum pump or water aspirator through a vacuum trap made from a 500-mL sidearm flask fitted with a one-hole stopper and glass tubing.

5.0 REAGENTS

5.1 Reagent-grade chemicals shall be used in all tests. Unless otherwise indicated, all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagents are of sufficiently high purity to permit their use without adversely affecting the accuracy of the determinations. For cleanup, use silica gel cartridges or loose silica gel.

5.2 Solvents -- Spectrophotometric grade, or equivalent.

5.2.1 Tetrachloroethylene, C_2Cl_4

5.3 Materials for the preparation of the reference oil mixture -- Spectrophotometric grade, or equivalent.

5.3.1 n-Hexadecane, $CH_3(CH_2)_{14}CH_3$

5.3.2 Isooctane, $(CH_3)_3CCH_2CH(CH_3)_2$

5.3.3 Chlorobenzene, C_6H_5Cl

5.4 Silica gel

5.4.1 Silica gel solid-phase extraction cartridges (40-μm particles, 60-Å pores), 0.5 g, Supelco, J.T. Baker, or equivalent.

5.4.2 Silica gel, 60 to 200 mesh, Davidson Grade 950, or equivalent (deactivated with 1 to 2 percent water).

5.5 Calibration mixtures

5.5.1 The material of interest, if available, or the same type of petroleum fraction, if it is known and original sample material is unavailable, shall be used for the preparation of calibration standards. Reference oil is to be used only for unknowns. Whenever possible, a GC fingerprint should be run on unknowns to determine the petroleum fraction type.

5.5.2 Reference oil -- Pipet 15.0 mL n-hexadecane, 15.0 mL isooctane, and 10.0 mL chlorobenzene into a 50-mL glass-stoppered bottle. Maintain the integrity of the mixture by keeping the bottle stoppered, except when withdrawing aliquots. Refrigerate at 4°C when not in use.

5.5.3 Stock standard -- Pipet 0.5 mL calibration standard (Section 5.8.1 or 5.8.2) into a tared 100-mL volumetric flask and stopper immediately. Weigh and dilute to volume with tetrachloroethylene.

5.5.4 Working standards -- Pipet appropriate volumes of stock standard (Section 5.5.3) into 100-mL volumetric flasks according to the cell size to be used. Dilute to volume with tetrachloroethylene. Calculate the concentrations of the standards from the stock standard concentrations.

5.6 Calibration mixture for silica gel cleanup -- Prepare a stock solution of corn oil by placing about 1 mL (0.5 to 1 g) of corn oil into a tared 100-mL volumetric flask. Stopper the flask and weigh to the nearest milligram. Dilute to the mark with tetrachloroethylene, and shake the contents to effect dissolution. Prepare additional dilutions to cover the range of interest.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Solid samples should be collected and stored as any other solid samples containing semivolatile analytes.

6.2 Samples should be analyzed with minimum delay, upon receipt in the laboratory, and must be kept refrigerated prior to analysis.

7.0 PROCEDURE

7.1 Prepare solid and sludge samples according to Method 3560.

7.2 Add 0.3 g of the loose silica gel to the 3-mL extract and shake the mixture for 5 minutes, or pass the extract through a 0.5-g silica gel solid-phase extraction cartridge (conditioned with 5 mL tetrachloroethylene). When working with loose silica gel, filter the extract through a plug of precleaned silanized glass wool in a disposable glass pipet.

7.3 After the silica-gel cleanup, fill a clean IR cell with the solution and determine the absorbance of the extract. If the absorbance exceeds the linear range of the IR spectrophotometer, prepare an appropriate dilution and reanalyze. The possibility that the absorptive capacity of the silica gel has been exceeded can be tested at this point by repeating the cleanup and determinative steps.

7.4 Select appropriate working standard concentrations and cell path lengths according to the following ranges:

Path length (mm)	Concentration range (μg/mL of extract)	Volume (mL)
10	5 to 500	3
50	1 to 100	15
100	0.5 to 50	30

Calibrate the instrument for the appropriate cells using a series of working standards. It is not necessary to add silica gel to the standards. Determine absorbance directly for each solution at the absorbance maximum at about 2950 cm^{-1}. Prepare a calibration plot of absorbance versus concentration of petroleum hydrocarbons in the working standards.

7.5 Determine the concentration of petroleum hydrocarbons in the extract by comparing the response against the calibration plot.

7.6 Calculate the concentration of TPHs in the sample using the formula:

$$\text{Concentration (mg/Kg)} = \frac{R \times D \times V}{W}$$

where:

R = mg/mL of TPHs as determined from the calibration plot
V = volume of extract in milliliters
D = extract dilution factor, if used
W = weight of solid sample in kilograms.

7.7 Recover the tetrachloroethylene used in this method by distillation or other appropriate technique.

8.0 QUALITY CONTROL

8.1 Reagent blanks or matrix-spiked samples must be subjected to the same analytical procedures as those used with actual samples.

8.2 Refer to Chapter One for specific Quality Control procedures and to Method 3500 for sample preparation procedures.

9.0 PRECISION AND ACCURACY

9.1 Table 1 presents a comparison of certified values and the values obtained using Methods 3560 and 8440. Data are shown for both Freon-113 and tetrachloroethylene, since both solvents were found to be acceptable as collection solvent. However, only tetrachloroethylene is recommended as collection solvent for TPHs in Method 3560.

9.2 Tables 2 and 3 present accuracy and precision data from the single-laboratory evaluation and the interlaboratory evaluation of Methods 3560 and 8440, respectively. These data were obtained by extracting samples at 340 atm/80°C/60 min dynamic (Table 2) or at 340 atm/80°C/30 min dynamic (Table 3).

10. REFERENCES

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2. Methods for Chemical Analysis of Water and Wastes; U.S. Environmental Protection Agency. Office of Research and Development, Environmental Monitoring and Support Laboratory. ORD Publication Offices of Center for Environmental Research Information, Cincinnati, OH, 1983; EPA-600/4-79-020.

3. Lopez-Avila, V., N. S. Dodhiwala, J. Benedicto, and R. Young, "SFE/IR Method for the Determination of Petroleum Hydrocarbons in Soils and Sediments," EPA 600/X-92/046, U. S. EPA, Environmental Monitoring Systems Laboratory, Las Vegas, NV, April 1992.

4. Lopez-Avila, V., R. Young, and R. Kim, "Interlaboratory Evaluation of an Off-Line SFE/IR Method for the Determination of Petroleum Hydrocarbons in Solid Matrices," EPA 600/X-93/XXX, U.S. EPA, Environmental Monitoring Systems Laboratory, Las Vegas, NV, January 1993.

TABLE 1. CERTIFIED AND SPIKE VALUES COMPARED WITH RESULTS OBTAINED BY METHODS 3560/8440

Reference Material	Spike level or certified level (mg/Kg)	Methods 3560/8440 (mg/Kg)
Environmental Resource Assoc.		
TPH-1 soil (Lot 91012)	1,830	1,920 ± 126[a]
Environmental Resource Assoc.		
TPH-2 soil (Lot 91012)	2,230	2,150 ± 380[a]
Clay soil spiked with kerosene	100	86.0; 93.0[b]
Clay soil spiked with light gas oil	100	84.0; 98.0[b]
Clay soil spiked with heavy gas oil	100	103; 108[b]
Clay soil spiked with medium neutral oil	100	125 ± 19.4[c]
Clay soil spiked with heavy neutral oil	100	126 ± 15.8[d]
Clay soil spiked with heavy lube oil	100	118; 155[b]
Environmental Resource Assoc.		
TPH-1 soil (Lot 91017)	614	562; 447[b]
Environmental Resource Assoc.		
TPH-2 soil (Lot 91017)	2,050	1,780; 1,780[b]
SRS103-100 soil	32,600	29,100 ± 1,930[e]

[a] Three 60-min extractions. The extracted materials were collected in Freon-113; the concentrations were determined against the reference oil standard.

[b] Duplicate 30-min extractions. The extracted materials were collected in tetrachloroethylene; the concentrations were determined against standards made from the spiking materials, except TPH-1 and TPH-2 soils where reference oil was used to determine concentrations.

[c] Six 30-min extractions. The extracted materials were collected in tetrachloroethylene; the concentrations were determined against standards made from the spiking material.

[d] Four 30-min extractions. The extracted materials were collected in tetrachloroethylene; the concentrations were determined against standards made from the spiking material.

[e] Three 30-min extractions. The extracted materials were collected in tetrachloroethylene; the concentrations were determined against the reference oil standard.

TABLE 2. SINGLE-LABORATORY METHOD ACCURACY AND PRECISION FOR METHODS 3560/8440 FOR SELECTED MATRICES

Matrix	Certified or spike value (mg/Kg)	Spike Material	Method accuracy (% recovery)	Method precision (% RSD)
Clay soil[a]	2,500	Motor oil	104	8.5
ERA TPH-1[a] (Lot 91016)	2,350	Vacuum oil	80.3	19.7
ERA TPH-2[a] (Lot 91016)	1,450	Vacuum oil	88.6	19.6
SRS103-100[b]	32,600	c	94.2	4.0

[a] Eight determinations were made using two different supercritical fluid extraction systems. The extracted materials were collected in Freon-113.

[b] Ten determinations were made using three different supercritical fluid extraction systems. The extracted materials were collected in Freon-113.

[c] This is a standard reference soil certified for polynuclear aromatic hydrocarbons. No spike was added.

TABLE 3. INTERLABORATORY METHOD ACCURACY AND PRECISION FOR METHOD 3560/8440 FOR SELECTED MATRICES

Compound name	True concentration (mg/Kg)	Mean concentration[a] (mg/Kg)	s_r[b] (mg/Kg)	s_R[c] (mg/Kg)	Percent RSD_r[d]	Percent RSD_R[e]	Percent mean recovery	Number of laboratories
Perkin-Elmer FTIR								
ERA TPH-1 soil	614	654	111	297	17.0	45.4	107	14
ERA TPH-2 soil	2,050	1,850	213	321	11.5	17.3	90.2	13
SRS103-100 soil	32,600	26,820	4,320	9,720	16.1	36.2	82.3	14
Clay soil spiked with motor oil	10,000	7,790	1,000	1,660	12.9	21.3	77.9	13
Buck-Scientific IR								
ERA TPH-1 soil	614	618	113	296	18.2	47.9	101	14
ERA TPH-2 soil	2,050	1,670	194	278	11.7	16.7	81.5	13
SRS103-100	32,600	24,750	3,740	8,650	15.1	35.0	75.9	14
Clay soil spiked with motor oil	10,000	8,180	910	1,500	11.1	18.3	81.8	13

[a] The number of replicates per laboratory was three.
[b] s_r - repeatability standard deviation.
[c] s_R - reproducibility standard deviation.
[d] RSD_r - repeatability relative standard deviation.
[e] RSD_R - reproducibility relative standard deviation.

APPENDIX C

LIST OF INSTRUCTIONS FOR COLLABORATORS

1~ 2~ 3~
4~
5~
6~

Dear 1~ 3~:

Thank you for participating in the EPA's interlaboratory study on extracting total petroleum hydrocarbons (TPHs) from environmental samples using supercritical fluid extraction (SFE). Its purpose is twofold: first, to verify SFE as a viable alternative to the currently approved extraction techniques for TPHs, and second, to establish a feasible non-CFC solvent for collecting the material that is extracted by the supercritical carbon dioxide.

Your package should contain the following:

1. One set of instructions.

2. Forms to record SFE conditions and observations.

3. Nine vials containing soil samples labeled Vials No. 1 through 9. Vials No. 1 through 3 contain at least 10 g soil each. Vials No. 4 through 9 contain 3.00 g soil each. Refrigerate all samples upon receipt.

4. Twenty empty vials with Teflon-lined screw caps for sending the extracts to MRI-California Operations.

5. An additional vial is included (labeled MRI# 000074) that contains anhydrous sodium sulfate (Mallinckrodt AR); anhydrous sodium sulfate is recommended for use with the sample contained in Vial No. 9.

If anything is missing or damaged, please contact me immediately for replacement. We made arrangements with Aldrich to send you tetrachloroethylene (HPLC-grade, Lot No. 06626TX) immediately. If the lot number differs from this number, do not use, but notify me so that the correct tetrachloroethylene can be sent to you. SFE-grade carbon dioxide from Scott Specialty Gases will also be sent to you. Please do not use other grades of carbon dioxide for this study.

After you have collected the 15 extracts, please send them to MRI-California Operations by March 27, 1992 for analysis by IR.

For questions or comments, do not hesitate to write, call, or fax a message to:

Richard Young
MRI-California Operations
625-B Clyde Avenue
Mountain View, CA 94043
Phone: (415) 694-7700
Fax: (415) 694-7983

For your information, there are 15 laboratories participating in the study. Our plans are to complete all IR analyses and the statistical analyses by the end of April 1992, and to present the results of the study at the 8th Annual Waste Testing and Quality Assurance Symposium in Washington, DC, in July 1992. Also, when the study is completed, we will finalize the method protocols (Method 3560 for the SFE and Method 8440 for the IR determination of TPHs) and send you a copy as soon as they are approved by EPA.

Sincerely,

MIDWEST RESEARCH INSTITUTE –
CALIFORNIA OPERATIONS

Richard Young
Senior Chemist

Enclosures

INSTRUCTIONS FOR THE EPA INTERLABORATORY STUDY ON SFE/IR METHOD FOR TPHs

Your Laboratory Code is: 15

REAGENTS

1. Tetrachloroethylene Aldrich Lot No. 06626TX. Please do not use any other grade of tetrachloroethylene for this study.

2. Sodium sulfate (anhydrous) known to be free of interferences in the C-H stretch band range 3200 to 2700 cm^{-1}

3. SFE-grade carbon dioxide from Scott Specialty Gases. Please do not use any other grade of carbon dioxide for this study.

APPARATUS

1. Supercritical fluid extraction system. Follow manufacturer's instructions on how to operate the system. Make sure that the extraction vessels and fittings are rated to at least 340 atm. Also make sure that the temperature is maintained during the extraction at 80°C.

2. Since tetrachloroethylene freezes when carbon dioxide is expanded into it , the collection vessel must be immersed in water of room temperature (20 to 25°C).

SAFETY

1. A safety feature to prevent overpressurization is required on the extraction system. This feature should be designed to protect the laboratory personnel and the instrument from possible injuries or damage resulting from equipment failure under high pressure.

2. Liquid carbon dioxide can cause "burns" because of its low temperature (-70°C).

3. The material safety data sheets (MSDS) for tetrachloroethylene and sodium sulfate should be reviewed before their use.

INTERFERENCES

1. The analyst must demonstrate through the analysis of system blanks that the SFE system is free from interferences. To do this, perform a simulated extraction, using an empty extraction vessel and a known amount of carbon dioxide under the same conditions as those that normally will be used for sample extraction, and analyze the extract by IR. The blank should have < 10 ppm TPHs (based on using approximately 30 mL of carbon dioxide and collecting the extract in 3 mL tetrachloroethylene). If you cannot perform the IR analysis of the system blank, send the extract for the system blank, labeled with your laboratory code, the term "blank", and the form recording SFE conditions and observations, to MRI—California Operations for analysis.

2. The extraction vessel(s), frits, restrictor(s), and selector valve may retain solutes. Therefore, it is good practice to clean the extraction system after each extraction. Replacement of the restrictor may be necessary when system blanks indicate carryover. At least one system blank should be performed daily when the instrument is in use. Furthermore, system blanks should be performed after each extraction of a high-concentration sample. If system blanks continue to indicate contamination even after replacement of the extraction vessel and the restrictor, the multiport valve must be cleaned.

3. Be aware of any organic solvents used to clean any parts of the extraction system or glassware since these may introduce interferents.

SAMPLE PREPARATION

Remove the sample vial from the refrigerator and follow the specific directions for each vial.

1. Vials 1 and 2: perform triplicate extractions using 3-g portions of this material. Weigh 3.00 g sample each time.

2. Vial 3: perform triplicate extractions using 3-g portions of this material. Due to the concentration and nature of this sample (may plug restrictors), it is suggested that it be extracted last.

3. Vials 4 though 8: extract the entire contents of the vial weighing the vial before and after emptying to verify that the vial contained 3.00 g sample. Record any differences from this amount.

4. Vial 9: extract the entire contents, weighing the vial before and after emptying to verify that the vial contained 3.00 g sample. Record any differences from this amount. Add 3 g sodium sulfate as a drying agent.

For each extraction, transfer the sample to a clean extraction vessel and use two plugs of silanized glass wool to hold the sample in place and to fill the void volume. Attach the end fittings and install the extraction vessel in the oven. Always use clean frits for each extraction vessel.

EXTRACTION

1. Fill the collection vessel with 3 mL tetrachloroethylene Aldrich Lot No. 06626TX. Do not use any other grade of tetrachloroethylene. Since tetrachloroethylene freezes when carbon dioxide is expanded into it, the collection vessel must be immersed in a beaker with water of room temperature (20 to 25°C).

2. Set the SFE pressure to 340 atm and the temperature to 80°C. Extract for 30 min at a flowrate of 1 to 2 mL/min (as liquid carbon dioxide).

3. Record extraction conditions and observations on the forms provided. Identify each extraction by vial number. When triplicate extractions are performed on the same sample, add the suffix A,

B, or C. That is, the extracts should be labeled 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B, 3C for matrices in vials 1, 2, and 3, respectively.

4. Transfer the tetrachloroethylene extract to one of the vials provided. Do not readjust the solvent volume back to 3 mL. The vial should be sealed tightly with the Teflon-lined cap. Label the vial in a legible and permanent manner in the following way:

   [15] - [extraction identification]

   For example, if your lab code is 15 and you have just extracted the third sample from Vial 2, the label should read "15-2C."

5. Store the extract in a refrigerator at 4°C until it can be shipped to MRI-California Operations.

EXTRACT SHIPMENT

After all of the extractions have been performed, ship the extracts in the vials provided to:

Richard Young
MRI-California Operations
625-B Clyde Avenue
Mountain View, CA 94043

Ship the extracts packed in dry ice via overnight carrier. Include the forms with the recorded SFE conditions and observations. Please ship so that receipt occurs during weekdays (i.e., do not send out on Friday). Fax a message indicating when you are sending the extracts. Please ship extracts back by March 27, 1992.

Your participation is highly appreciated, and we look forward to receiving your extracts to make this a successful study. We also welcome your comments and suggestions concerning this study.

FORM I: INSTRUMENT OPERATING CONDITIONS FOR SUPREX, ISCO, DIONEX-LEE SCIENTIFIC, AND CCS EXTRACTION SYSTEMS[a]

Instrument: _____ Operator: _____ Date: _____

Supercritical fluid: _____ Supplier: _____ Grade: _____

Modifier: _____ Percent modifier: _____

Extraction vessel volume (mL): _____ Number: _____

Extraction vessel dimensions: _____ ID x _____ length (mm)

Extraction vessel position: Horizontal ___ Vertical ___

Restrictor: _____ ID x _____ OD x _____ length (mm)

Collection system:
Vial size: _____
Manufacturer: _____
Catalog number: _____
Solvent: _____ Volume: _____ Initial
_____ Final

Operating conditions:

Dynamic or static	_____
Pressure (atm)	_____
Temperature (°C)	_____
Flowrate (mL/min)	_____
Density (g/mL)	_____
Time (min)	_____
Restrictor temp. (°C)	_____
Volume of CO_2 (mL)	_____

[a] If conditions vary for different samples, submit separate form.

FORM II: INSTRUMENT OPERATING CONDITIONS FOR HP MODEL 7680A EXTRACTOR

SAMPLE HISTORY

Original entry:
Last update:

SAMPLE INFORMATION

Sample name:
Sample ID:
Operator:
Sample amount:
Comments:

EXTRACTION STEP

FLUID DELIVERY

Density:	g/mL
Pressure:	bars (convert bars to atm)
Flowrate:	mL/min
Extraction fluid:	CO_2

EXTRACTION CHAMBER

Chamber temperature:	°C
Equilibration time:	min
Extraction time:	min
Thimble size:	mL
Thimble volumes swept:	

ANALYTE TRAP

Analyte:	Intermediate volatile
Trap material:	
Nozzle temperature:	°C
Trap temperature:	°C

FORM II: INSTRUMENT OPERATING CONDITIONS FOR HP MODEL 7680A EXTRACTOR (continued)

FRACTION OUTPUT

Rinse substep	Solvent name	Volume (mL)	Rate (mL/min)	Nozzle temperature	Trap temperature	Vial number